ALUMINUM COMPOUNDS VOL I #43

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VOLUME 1

GRAS MONOGRAPH SERIES

ALUMINUM COMPOUNDS

prepared for
THE FOOD AND DRUG ADMINISTRATION
DEPARTMENT OF HEALTH, EDUCATION
AND WELFARE

JUNE 8, 1973

prepared by

Tracor Jitco, Inc.

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BIOCHEMICAL SECTION

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SUMMARY

Description and Specifications

Aluminum potassium sulfate [$AlK(SO_4)_2$] and its dodecahydrate occurs as an odorless, sweetish, white crystal powder or as large, hard transparent crystals or crystalline fragments of molecular weight 258.20 (anhydrous). One gram is soluble in 7.2 ml of water and 0.3 ml of boiling water; it is freely soluble in glycerol but insoluble in alcohol. The Food Chemicals Codex (034) specifies that food grade potassium alum be not less than 99.5% of $AlK(SO_4)_2 \cdot 12H_2O$.

Aluminum sodium sulfate [$AlNa(SO_4)_2$] and its dodecahydrate occurs as colorless crystals, or as white granules or powder of molecular weight 242.09 (anhydrous). It is odorless with a saline, astringent taste. The anhydrous form is slowly soluble in water while the dodecahydrate is freely soluble. Both forms are insoluble in alcohol. The anhydrous crystals have a melting point of 61°C. The Food Chemicals Codex (034) specifies that the anhydrous form of food grade sodium alum be not less than 96.5% $AlNa(SO_4)_2$ after drying, with not more than 10% loss on drying; the dodecahydrate form should be not less than 99.5% $AlNa(SO_4)_2$ after drying with not more than 47.2% loss on drying.

Aluminum ammonium sulfate [$AlNH_4(SO_4)_2$] and its dodecahydrate occurs as large colorless crystals, white granules, or powder of molecular weight 237.14 (anhydrous). It is odorless and has a styptic taste. The melting point of the anhydrous form is 94.5°C. One gram is soluble in 7 ml of water, 0.5 ml of boiling water, is freely soluble in glycerol and insoluble in alcohol. Solutions are acid to litmus. The Food Chemicals Codex (034) specifies that food grade ammonium alum be not less than 99.5% $AlNH_4(SO_4)_2 \cdot 12H_2O$.

Aluminum sodium phosphate, $NaAl_3H_{14}(PO_4)_8 \cdot 4H_2O$ (molecular weight 949.88) or $Na_3Al_2H_{15}(PO_4)_8$ (molecular weight 897.82) occurs as a white, odorless powder which is insoluble in water, but soluble in hydrochloric acid. The Food Chemicals Codex (034) specifies that food grade aluminum sodium phosphate be not less than 95.0% $NaAl_3H_{14}(PO_4)_8 \cdot 4H_2O$ with loss on ignition between 19.5 and 21%; or not less than 95% $Na_3Al_2H_{15}(PO_4)_8$ with loss on ignition between 15 and 16%.

Aluminum sulfate [$Al_2(SO_4)_3$], containing up to 18 molecules of water of crystallization, occurs as white, lustrous crystals, crystalline fragments, or powder. Though it normally occurs as the octadeca-

hydrate, the article of commerce usually contains 5 to 10% less water than the theoretical compound. The Food Chemicals Codex (034) specifies that the anhydrous form of food grade aluminum sulfate be not less than 99.5% $Al_2(SO_4)_3$, calculated on the ignited basis; and that the octadecahydrate form be not less than 99.5% and not more than the equivalent of 114.0% of $Al_2(SO_4)_3 \cdot 18H_2O$ (corresponding to not more than approximately 101.7% of $Al_2(SO_4)_3 \cdot 14H_2O$).

Sodium aluminate ($AlNaO_2$) occurs as white, granular mass which is very soluble in water and insoluble in alcohol. Aqueous solutions are strongly alkaline. The molecular weight is 81.97 and the melting point is 1650°C.

Aluminum oleate ($C_{54}H_{99}AlO_6$), molecular weight 871.30, occurs as a yellowish, viscid mass which is insoluble in water, soluble in alcohol, benzene, ether, and oil of turpentine.

Aluminum palmitate ($C_{48}H_{93}AlO_6$), molecular weight 793.25, occurs as a white to yellow mass or powder. It is practically insoluble in water and alcohol, but, when fresh, it is soluble in petroleum ether and turpentine.

Aluminum hydroxide [$Al(OH)_3$], molecular weight 77.99, occurs as a white, viscous suspension, or a white, bulky amorphous powder. It is practically insoluble in water, but is soluble in aqueous alkaline solutions, or in HCl, H_2SO_4, and other strong acids in the presence of some water.

Sodium phosphoaluminate. No description or specification information available.

Acute Toxicity

Aluminum nitrate

The following lethal doses have been established for intraperitoneal administration: LD_{10} mouse, 213 mg/kg; rat, 240 mg/kg. LD_{50} mouse, 320 (282-374) mg/kg; rat, 327 (283-378) mg/kg (Hart and Adamson, 069).

Aluminum chloride

The following lethal doses measured in mg Al/kg have been determined for peroral administration: LD_{50} in mice: 770 $\pm$ 120 (Ondreicka *et al.*, 134); LD_{100} in rats: 1100 (Berlyne *et al.*, 014).

The LD_{100} in mg/kg BW has been determined by subcutaneous administration as: rat, 7000-8000 (Underhill *et al.*, 185); guinea pig, 5000-7000 (Underhill *et al.*, 185); rabbit, 7000-8000 (Underhill *et al.*, 185).

Aluminum sulfate

The following lethal doses measured in mg Al/kg have been determined perorally: LD_{50} mice: 980 $\pm$ 90 (Ondreicka *et al.*, 134); LD_{100} rat: 1100.

The LD_{100} determined subcutaneously for rabbits is 7000-8000 mg/kg BW (Underhill *et al.*, 185).

Aluminum hydroxide

The LD_{100} in mg Al/kg/day administered intraperitoneally to rats was determined as 150 (Berlyne et al., 014).

Short-Term Studies

Whether or not ingested aluminum salts are toxic to animals has been studied for about 70 years, since the question of the safety of using aluminum cooking utensils was first raised. More recently, the widespread therapeutic use of aluminum hydroxide as an antacid in the treatment of gastric ulcers has again resulted in much controversy about the potential toxicity of this metal to humans.

Schaeffer and co-workers (156) described very definite inflammatory lesions in the gastrointestinal mucous membrane of both mice and dogs after prolonged ingestion of special breads leavened with alum or alum-phosphate baking powders. They were of the opinion that the presence of aluminum chloride in the stomach, even in as small amounts as would be present from the daily ingestion of aluminum-containing baking powders, might give rise to gastric and duodenal ulcers.

Ondreicka et al. (134) claimed that earlier studies which showed no toxic effects from the ingestion of low doses of an aluminum salt were incorrect because of their lack of sensitivity. They found retardation of growth in litters of mice subsequent to the first

litter, which was fed low doses of aluminum chloride. The authors point out that the failure to find any abnormalities in tissue morphology or the blood indicates the necessity for seeking more sensitive indicators of metabolic disturbances resulting from chronic ingestion of small amounts of aluminum compounds.

Jones (082) produced a marked rickets accompanied by low serum phosphate in rats fed basic aluminum acetate or aluminum sulfate. Nearly one-third the skeletal mineral contents were lost.

Recently Berlyne et al. (014) showed that low doses of aluminum compounds (aluminum sulfate, chloride, and hydroxide) can be harmful to rats, particularly those with renal damage. They caution that in the light of recent studies, the widespread use of aluminum salts in man should be suspended until further studies are carried out.

Several researchers have produced dystrophy and death in chickens by feeding aluminum compounds. Deobold and Elvehjem (039) used a soluble aluminum salt, aluminum phosphate. The bone ash and blood phosphorus were severely reduced. Deleterious effects were avoided by adding an amount of sodium acid phosphate sufficient to unite with the Al in the diet.

Williams and co-workers (196 and 176) and Pragay (140) found that aluminum hydroxide gel fed to chickens

also caused dystrophy and the higher the dose, the greater the mortality. Both groups of researchers found lowered plasma levels of Vitamin A.

Seibert and Wells (162) found that sodium aluminum sulfate and aluminum chloride fed to rabbits resulted in a marked decrease in hemoglobin and erythrocyte count. The authors conclude that ingesting small daily doses of aluminum compounds in any form (even compounds as presumably inert as aluminum hydroxide) produces changes in the blood and tissues, indicating absorption through the intestinal tract. They suggest that in a manner analogous to lead poisoning, years of slow absorption are required to effect the same results as are found by direct injection into the blood stream.

In humans, ingestion of aluminum hydroxide has been found to cause intestinal obstruction in seriously ill patients (Havens, 071). Bloom and Flinchum (019) have found evidence of osteomalacia produced by ingesting large amounts of aluminum hydroxide. They are concerned that this is going unnoticed. Lotz et al. (109) found that aluminum hydroxide can impair phosphorus absorption in man. All subjects fed aluminum hydroxide daily over several months developed debility and the syndrome of phosphorus depletion. The authors conclude that this clinically important syndrome, which can be counteracted by providing adequate dietary phosphorus, is the direct

result of prolonged and excessive ingestion of aluminum-containing antacids.

Special Studies

Berlyne et al. (013) found that the ingestion of aluminum hydroxide by patients with advanced renal failure elevated their serum aluminum levels excessively. The authors caution that since the toxic effects of this hyperaluminemia are as yet unknown, the use of aluminum salts in renal failure should be regarded with some concern.

Erdohazi and Newman (043) describe several cases of granuloma in humans produced by vaccines containing aluminum compounds.

Absorption and Distribution

Ondreicka et al. (134) point out that it is only recently that the analytical methods for determination of aluminum have become sensitive enough to adequately detect its presence in tissues. In a study with rats, Ondreicka et al. (134) demonstrated increased retention of aluminum when high doses of aluminum sulfate were ingested. Significant amounts of aluminum were found, particularly in the liver, testes, and bone. The authors conclude that the concentration of aluminum in the body is a function of the amount of aluminum ingested.

Ondreicka et al. (133) found in a subsequent study that the retention of aluminum is related to the food composition. When aluminum and fluorine (as

calcium fluoride) were administered simultaneously, there was a decreased aluminum level in all the organs. The authors suggest that the aluminum is removed from the tissues by the formation of an aluminum fluoride complex which is more soluble than calcium fluoride.

Underhill and Peterman (184) found that when dogs were fed large amounts of aluminum salts over a long period, the average aluminum content of the blood decreased. Absorbed aluminum was highest in the spleen, brain, liver, and kidney. The authors noted with surprise the high amount of aluminum in the thyroid.

The same authors found that in feeding studies with humans using sodium aluminum phosphate baking powder, there appeared to be a delay in the absorption of aluminum after eating aluminum-rich food. They speculate that this may indicate that aluminum is more easily absorbed from the lower than the upper part of the intestinal tract. The observation that the aluminum content of urine tends to increase after ingestion of aluminum-rich food indicates that aluminum must be present at times in the blood even if not always detected.

Metabolism and Excretion

Aluminum has been found by a number of researchers to disturb phosphorus metabolism. The disturbance of phosphorylating mechanisms in turn affects ATP production and carbohydrate metabolism.

Ondreicka et al. (134) using labeled phosphorus (^{32}P) demonstrated that ingesting aluminum salts reduces phosphorus absorption. Furthermore, they found that there was a decrease in the incorporation of ^{32}P into the phospholipid fraction as well as into the ribonucleic and deoxyribonucleic acid in various tissues. Additional evidence for the disturbance of phosphorylating mechanisms by aluminum intoxication was the decrease in rat serum adenosine triphosphate while adenosine mono- and di-phosphates increased. The authors point out that decreased production of ATP could endanger the course of a whole series of phosphorylation reactions, such as the synthesis of phospholipids and nucleic acids.

Vozar (190) found that orally ingested aluminum compounds impair the sequence of biochemical processes in the glycide metabolism of rats, as seen in changes in the glycide reserves. The author attributes this metabolic disturbance to a malfunction in phosphorus metabolism caused by ingesting aluminum salts.

Kortus (100) also found disturbances in glycide metabolism in rats after ingestion of an aluminum salt.

Liver glycogen decreased and lactic and pyruvic acids increased, indicating that there was a decreased absorption of glucose from the gut.

Child (029) found that after ingesting aluminum hydroxide a patient with chronic duodenal ulcer developed painful concretions composed largely of aluminum salts of fatty acids.

Bailey et al. (006) observed that patients with chronic renal failure absorbed and retained aluminum ingested as aluminum hydroxide. Plasma phosphorus fell in all patients studied. The authors explain their observations by suggesting that after absorption aluminum combines with phosphate and is deposited in bone.

Effect on Enzymes and Other Parameters

Berlyne et al. (014) showed that ingesting aluminum compounds (aluminum sulfate and hydroxide) depresses rat liver respiration indicating a direct toxic action on the liver cell. Liver protein concentration was reduced in rats ingesting aluminum hydroxide, which the authors suggest may be due to direct interference with protein synthesis.

Street (177) found that when a soluble form of aluminum is fed to rats, in amounts nearly equal to dietary phosphorus, almost complete precipitation of phosphorus occurs in the intestinal tract. The less soluble aluminum hydroxide is partially converted to

aluminum chloride in the gastrointestinal tract, which accounts for its reaction with intestinal phosphate.

Bishop et al. (018) found that chickens fed aluminum hydroxide gel became dystrophic and had markedly reduced ATP levels in their blood. They attribute the muscle weakness to reduced ATP levels in muscle, owing to a general failure of phosphorylation since the erythrocytes cannot maintain their ATP levels and thus there is a decreased availability of inorganic phosphate. When inorganic phosphate is supplied, partial recovery occurs.

Schwab (160) has found that aluminum compounds affect certain hormones. When small subcutaneous doses of aluminum chloride are given to rabbits, the hypoglycemic effect of insulin is strengthened and prolonged, while strong doses inhibit the hormone. A similar effect was observed for the hypoglycemic effect of adrenaline.

Fauley et al. (051) found that ingestion of aluminum hydroxide interferes with phosphorus retention in both dogs and humans.

Drug Interaction

Wegria et al. (194) did not find any modification of serum salicylate level in human subjects who ingested aluminum hydroxide with doses of aspirin.

Consumer Exposure

In addition to varying amounts of aluminum naturally present in foods, the average American may consume substantial amounts of aluminum sodium sulfate (1494 mg av.) and sodium aluminum phosphate (1640 mg) in baked goods in his daily diet. Other aluminum salts are added to meat products, relishes, and cheese in amounts that give a possible average daily intake of 10 mg aluminum ammonium sulfate, 0.0739 mg aluminum potassium sulfate, and 246 mg aluminum sulfate (128).

Campbell et al. in 1957 (025) estimated that 10 to 100 mg was the average daily dietary intake for an adult human. This figure includes the aluminum that might be ingested from the preparation of food in aluminum cooking utensils. Not taken into consideration is the aluminum absorbed from airborne particulate matter as a consequence of the presence of aluminum in soil or from aluminum compounds used in the treatment of drinking water.

No figures were available on the amount of aluminum hydroxide ingested in antacid preparations. But as mentioned in another part of the summary, Bloom and Flinchum (019) found osteomalacia in a woman who had been daily dosing herself with an antacid preparation.

CHEMICAL INFORMATION

ALUMINUM POTASSIUM SULFATE

I. Nomenclature

A. Common names: Alum, Kalanite, Potassium alum

B. Chemical names: Aluminum potassium sulfate, dodecahydrate

Sulfuric acid, aluminum potassium salt(2:1:1), dodecahydrate

C. Trade names: none

D. Chemical Abstracts Services Unique Registry Number:

1004367 (anhydrous)

7784249 (dodecahydrate)

II. Empirical Formula

$AlKO_8S_2$ ($\cdot 12H_2O$)

III. Structural Formula

$AlK(SO_4)_2$ ($\cdot 12H_2O$)

IV. Molecular Weight: 258.20 (anhydrous), 474.38 (dodecahydrate)

V. Specifications

A. The Food Chemicals Codex Second Edition (034) presents the following food grade specifications for potassium alum:

1. Description

Large, transparent crystals or crystalline fragments, or a white crystalline powder. It is odorless and has a sweetish, astringent taste. One gram dissolves in 7.5 ml of water at 25° and in about 0.3 ml of boiling water. It is insoluble in alcohol, but is

freely soluble in glycerin. Its solutions are acid to litmus. A 1 in 20 solution gives positive tests for aluminum, for potassium, and for sulfate.

2. Specifications

Assay. Not less than 99.5% of $AlK(SO_4)_2 \cdot 12H_2O$.

Limits of impurities

Ammonium salts. Passes test.

Arsenic (as As). Not more than 3 parts per million (0.0003%).

Fluoride. Not more than 30 parts per million (0.003%).

Heavy metals (as Pb). Not more than 20 parts per million (0.002%).

Lead. Not more than 10 parts per million (0.001%).

Selenium. Not more than 30 parts per million (0.003%).

3. Tests

Assay. Weigh accurately about 1 g, dissolve it in 50 ml of water, add 50.0 ml of 0.05 M disodium ethylenediaminetetraacetate, and boil gently for 5 minutes. Cool, and add in the order given and with continuous stirring 20 ml of pH 4.5 buffer solution (77.1 g of ammonium acetate and 57 ml of glacial acetic acid in 1000 ml of solution), 50 ml of alcohol, and 2 ml of dithizone. Titrate with 0.05 M zinc sulfate to a bright rose-pink color, and perform a blank determination. Each ml of 0.05 M disodium ethylenediaminetetraacetate is equivalent to 23.72 mg of $AlK(SO_4)_2 \cdot 12H_2O$.

Ammonium salts. Heat 1 g with 10 ml of sodium hydroxide T.S. on a steam bath for 1 minute. The odor of ammonia is not perceptible.

Arsenic. A solution of 1 g in 35 ml of water meets the requirements of the Arsenic Test.

Heavy metals. Dissolve 1 g in 20 ml of water, add a few drops of diluted hydrochloric acid T.S., and evaporate to dryness in a porcelain dish. Treat the residue with 20 ml of water, and add 50 mg of hydroxylamine hydrochloride. Heat on a steam bath for 10 minutes, cool, and dilute to 25 ml with water. This solution meets the requirements of the Heavy Metals Test, using 20 µg of lead ion (Pb) and 50 µg of hydroxylamine hydrochloride in the control (Solution A).

Fluoride. Determine as directed in the test for fluoride under Aluminum Ammonium Sulfate.

Lead. A solution of 1 g in 10 ml of water meets the requirements of the Lead Limit Test, using 10 µg of lead ion (Pb) in the control.

Selenium. A solution of 2 g in 40 ml of dilute hydrochloric acid (1 in 2) meets the requirements of the Selenium Limit Test.

Packaging and storage. Store in well-closed containers.

Functional use in foods. Buffer; neutralizing, firming agent.

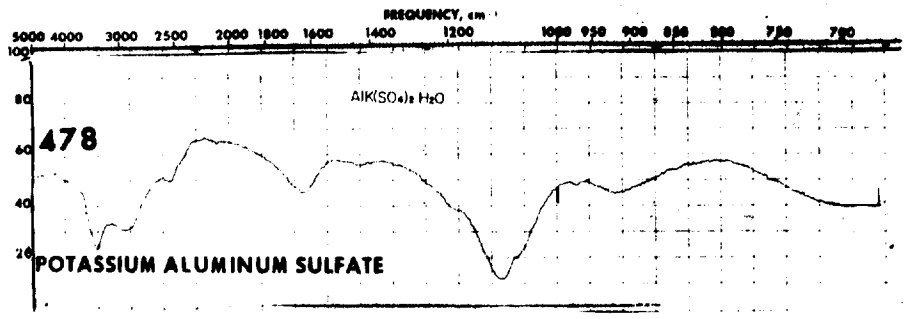
VI. Description

A. General characteristics:

Potassium alum exists as large, hard, transparent crystals or crystal fragments, or as a white crystal powder. It is odorless and has a sweetish astringent taste.

B. Physical properties:

The density of potassium alum is 1.725 and it has a melting point of 92.5°C. One gram is soluble in 7.2 ml of water (0.3 ml of boiling water), is freely soluble in glycerol, and is insoluble in alcohol. The I.R. spectra for potassium aluminum sulfate are shown below (151) (solvent: KBr).



C. Stability in containers:

Stable at ordinary temperatures; loses $9H_2O$ when kept for long periods of time at 60-65°C (or over H_2SO_4) which is reabsorbed on exposure to air.

ALUMINUM SODIUM PHOSPHATE

I. Nomenclature

A. Common names: none available

B. Chemical names: Aluminum sodium phosphate, acidic

Phosphoric acid, aluminum sodium salt

C. Trade names: none

D. Chemical Abstracts Unique Services Registry Number: 7785888

II. Empirical Formula

(a) $Al_3H_{15}NaO_{32}P_8 \cdot 4H_2O$

(b) $Al_2H_{15}Na_3O_{32}P_8$

III. Molecular Formula

(a) $NaAl_3H_{14}(PO_4)_8 \cdot 4H_2O$

(b) $Na_3Al_2H_{15}(PO_4)_8$

IV. Molecular Weight: 949.88 (form a); 897.82 (form b)

V. Specifications

A. The Food Chemicals Codex Second Edition (034) presents the following food grade specifications for sodium aluminum phosphate, acidic:

1. Description

A white, odorless powder. It is insoluble in water, but is soluble in hydrochloric acid. A 1 in 10 solution in dilute hydrochloric acid (1 in 2) gives positive tests for aluminum, and for phosphate, and it responds to the flame test for sodium.

2. Specifications

Assay. Not less than 95.0% of $NaAl_3H_{14}(PO_4)_8 \cdot 4H_2O$, or not less than 95.0% of $Na_3Al_2H_{15}(PO_4)_8$.

Loss on ignition. $NaAl_3H_{14}(PO_4)_8 \cdot 4H_2O$, between 19.5 and 21%; $Na_3Al_2H_{15}(PO_4)_8$, between 15 and 16%.

Neutralizing value. Not less than 100.

Limits of impurities.

Arsenic (as As). Not more than 3 parts per million (0.0003%).

Fluoride. Not more than 25 parts per million (0.0025%).

Heavy metals (as Pb). Not more than 40 parts per million (0.004%).

Lead. Not more than 10 parts per million (0.001%).

3. Tests

Assay. Transfer about 2.5 g, accurately weighed, into a 250-ml volumetric flask, add 15 ml of hydrochloric acid and one glass bead, and boil gently for about 5 minutes. Cool, dilute to volume with water, and mix. Transfer 10.0 ml of this solution to a 250-ml beaker, add phenolphthalein, and neutralize with ammonia. Add dilute hydrochloric acid (1 in 2) until the precipitate just dissolves, then dilute to 100 ml with water and heat to 70°-80°. Add 10 ml of 8-hydroxyquinoline and sufficient ammonium acetate until a yellow precipitate forms, then add 30 ml in excess. Digest at 70° for 30 minutes, filter through a previously dried and weighed Gooch crucible, and wash thoroughly with hot water. Dry at 105° for 2 hours, cool, and weigh. Each mg of the precipitate so obtained corresponds to 0.689 mg of $NaAl_3H_{14}(PO_4)_8 \cdot 4H_2O$, or to 0.977 mg of $Na_3Al_2H_{15}(PO_4)_8$.

Loss on ignition. Ignite at 750° to 800° for 2 hours.

Neutralizing value. Transfer 840.1 mg, accurately weighed, into a 375-ml casserole, and add 20 g of sodium chloride, 5 ml of

sodium citrate solution (1 in 10), and 25 ml of water. Stir vigorously at once for about 30 seconds, then add exactly 120 ml of 0.1 N sodium hydroxide, bring the suspension to a boil in exactly 2 minutes, and boil for 5 minutes. While the solution is still boiling hot, add exactly 0.05 ml of phenolphthalein, and titrate the excess alkali with 0.2 N hydrochloric acid until the pink color has almost disappeared. Boil the solution for 1 minute, and titrate again with 0.2 N hydrochloric acid until the pink color just disappears. Calculate the neutralizing value, as parts of $NaHCO_3$ equivalent to 100 parts of sodium aluminum phosphate, by the formula $120 - 2V$, in which V is the total volume of 0.2 N hydrochloric acid consumed.

Arsenic. A solution of 1 g in 10 ml of dilute hydrochloric acid (1 in 2) meets the requirements of the Arsenic Test.

Fluoride. Weigh accurately 2.0 g, and proceed as directed in the Fluoride Limit Test.

Heavy metals. Dissolve 500 mg in 2.5 ml of diluted hydrochloric acid T.S., and add water to make 25 ml. This solution meets the requirements of the Heavy Metals Test, using 20 µg of lead ion (Pb) in the control (Solution A).

Lead. A solution of 1 g in 5 ml of diluted hydrochloric acid meets the requirements of the Lead Limit Test, using 10 µg of lead ion (Pb) in the control.

Packaging and storage. Store in well-closed containers.

Functional use in foods. Leavening agent.

VI. Description

A. General characteristics:

Sodium aluminum phosphate occurs as a white odorless powder.

B. Physical properties:

Sodium aluminum phosphate is insoluble in water, but is soluble in hydrochloric acid.

C. Stability in containers:

Not available.

ALUMINUM SULFATE

I. Nomenclature

A. Common names: Cake alum, Patent alum

(occurs naturally as the mineral alunogenite)

B. Chemical names: Aluminum sulfate

Sulfuric acid, aluminum salt(3:2)

C. Trade names: none

D. Chemical Abstracts Services Unique Registry Number:

10043013 (anhydrous)

II. Empirical Formula

. $Al_2O_{12}S_3$ (anhydrous)

III. Structural Formula

$Al_2(SO_4)_3$ ($\cdot x H_2O$)

(x is normally 18, the octadecahydrate. The article of commerce, however, usually contains 5-10% less water than the theoretical compound).

IV. Molecular Weight: 342.15 (anhydrous)

V. Specifications

A. The Food Chemicals Codex Second Edition (034) presents the following food grade specifications for aluminum sulfate:

1. Description

Aluminum sulfate is anhydrous or contains 18 molecules of water of crystallization. Due to efflorescence, the hydrate may have a composition approximating the formula $Al_2(SO_4)_3 \cdot 14H_2O$. It occurs as a white powder, as shining plates, or as crystalline fragments. It is odorless and has a sweet taste, becoming mildly astringent. One gram of the hydrate dissolves in about 2 ml of

water. The anhydrous product approaches the same solubility, but the rate of solution is so slow that it initially appears to be relatively insoluble. The pH of a 1 in 20 solution is 2.9 or above. A 1 in 10 solution gives positive tests for aluminum, and for sulfate.

2. Specifications

Assay. $Al_2(SO_4)_3$ (anhydrous), not less than 99.5% of $Al_2(SO_4)_3$, calculated on the ignited basis; $Al_2(SO_4)_3 \cdot 18H_2O$ (hydrate), not less than 99.5% and not more than the equivalent of 114.0% of $Al_2(SO_4)_3 \cdot 18H_2O$, corresponding to not more than approximately 101.7% of $Al_2(SO_4)_3 \cdot 14H_2O$.

Limits of impurities.

Alkalies and alkaline earths. Passes test (about 0.4%).

Ammonium salts. Passes test.

Arsenic (as As). Not more than 3 parts per million (0.0003%).

Heavy metals (as Pb). Not more than 40 parts per million (0.004%).

Lead. Not more than 10 parts per million (0.001%).

Loss on ignition. $Al_2(SO_4)_3$ (anhydrous), not more than 5%.

[Note: This specification does not apply to $Al_2(SO_4)_3 \cdot 18H_2O$.]

Selenium. Not more than 30 parts per million (0.003%).

3. Tests

Assay. Weigh accurately an amount of sample equivalent to about 4 g of $Al_2(SO_4)_3$, transfer into a 250-ml volumetric flask, dissolve in water, dilute to volume with water, and mix. Pipet 10 ml of this solution into a 250-ml beaker, add 25.0 ml of 0.05 M disodium ethylenediaminetetraacetate, and boil gently for 5 minutes.

Cool, and add in the order given and with continuous stirring 20 ml of pH 4.5 buffer solution (77.1 g of ammonium acetate and 57 ml of glacial acetic acid in 1000 ml of solution), 50 ml of alcohol, and 2 ml of dithizone. Titrate with 0.05 M zinc sulfate until the color changes from green-violet to rose-pink, and perform a blank determination, substituting 10 ml of water for the sample. Each ml of 0.05 M disodium ethylenediaminetetraacetate is equivalent to 8.554 mg of $Al_2(SO_4)_3$ or to 16.67 mg of $Al_2(SO_4)_3 \cdot 18H_2O$.

Alkalies and alkaline earths. To a boiling solution of 2 g in 150 ml of water add a few drops of methyl red, and then add ammonia until the color of the solution just changes to a distinct yellow. Add hot water to restore the original volume, and filter while hot. Evaporate 75 ml of the filtrate to dryness, and ignite to constant weight. Not more than 4 mg of residue remains.

Ammonium salts. Heat 1 g with 10 ml of sodium hydroxide on a steam bath for 1 minute. The odor of ammonia is not perceptible.

Arsenic. A solution of 1 g in 35 ml of water meets the requirements of the Arsenic Test.

Heavy metals. Dissolve 500 mg in 20 ml of water, add a few drops of diluted hydrochloric acid, and evaporate to dryness in porcelain dish. Treat the residue with 20 ml of water, and add 50 mg of hydroxylamine hydrochloride. Heat on a steam bath for 10 minutes, cool, and dilute to 25 ml with water. This solution meets the requirements of the Heavy Metals Test, using 20 µg of lead ion (Pb) and 50 mg of hydroxylamine hydrochloride in the control (Solution A).

Lead. A solution of 1 g in 10 ml of water meets the requirements of the Lead Limit Test, using 10 µg of lead ion (Pb) in the control.

Loss on ignition. Weigh accurately about 2 g of $Al_2(SO_4)$ (anhydrous), and ignite, preferably in a muffle furnace, at about 500° for 3 hours. [Note: This test does not apply to $Al_2(SO_4)_3 \cdot 18H_2O$.]

Selenium. A solution of 2 g in 40 ml of dilute hydrochloric acid (1 in 2) meets the requirements of the Selenium Limit Test.

Packaging and storage. Store in well-closed containers.

Functional use in foods. Firming agent.

VI. Description

A. General characteristics:

Aluminum sulfate (containing up to 18 molecules of water of crystallization) occurs as white, lustrous crystals, crystalline fragments, or powder.

B. Physical properties:

Aluminum sulfate is soluble in 1 part of water and practically insoluble in alcohol. The density of the hydrate form is 1.61 and the density of the anhydrous form is 2.71.

C. Stability in containers:

Not available.

ALUMINUM SODIUM SULFATE

I. Nomenclature

A. Common names: Soda alum, sodium alum

B. Chemical names: Aluminum sodium sulfate, dodecahydrate

Sulfuric acid, aluminum sodium salt(2:1:1), dodecahydrate

C. Trade names: none

D. Chemical Abstracts Unique Services Registry Number:

7784283 (dodecahydrate)

II. Empirical Formula

$AlNaO_8S_2$ $(\cdot 12H_2O)$

III. Structural Formula

$AlNa(SO_4)_2$ $(\cdot 12H_2O)$

IV. Molecular Weight: 242.09 (anhydrous); 458.29 (dodecahydrate)

V. Specifications

A. The Food Chemicals Codex Second Edition (034) presents the following food grade specifications for sodium alum:

1. Description

Aluminum sodium sulfate is anhydrous or may contain up to 12 molecules of water of hydration. It occurs as colorless crystals, or white granules or powder. It is odorless and has a saline, astringent taste. The anhydrous form is slowly soluble in water. The dodecahydrate is freely soluble in water, and effloresces in air. Both forms are insoluble in alcohol. It responds to the flame test for sodium, and gives positive tests for aluminum, and for sulfate.

2. Specifications

Assay. Anhydrous form, not less than 96.5% of $AlNa(SO_4)_2$ after drying; dodecahydrate, not less than 99.5% of $AlNa(SO_4)_2$ after drying.

Loss on drying. Anhydrous form, not more than 10%; dodecahydrate, not more than 47.2%.

Neutralizing value. Anhydrous form, between 103 and 107.

Limits of impurities.

Ammonium salts. Passes test.

Arsenic (as As). Not more than 3 parts per million (0.0003%).

Fluoride. Not more than 30 parts per million (0.003%).

Heavy metals (as Pb). Not more than 20 parts per million (0.002%).

Lead. Not more than 10 parts per million (0.001%).

Selenium. Not more than 30 parts per million (0.003%).

3. Tests

Assay. Weigh accurately about 500 mg of a sample previously dried as directed in the test for Loss on drying, moisten with 1 ml of acetic acid, and dissolve it in 50 ml of water, warming gently on a steam bath until solution is complete. Cool, neutralize with ammonia T.S., add 50.0 ml of 0.05 M disodium ethylenediaminetetraacetate, and boil gently for 5 minutes. Cool, and add in the order given and with continuous stirring 20 ml of pH 4.5 buffer solution (77.1 g of ammonium acetate and 57 ml of glacial acetic acid in 1000 ml of solution), 50 ml of alcohol, and 2 ml of dithizone T.S. Titrate with 0.05 M zinc sulfate to a bright rose-pink color, and perform a blank determination. Each ml of

0.05 M disodium ethylenediaminetetraacetate is equivalent to 12.10 mg of $AlNa(SO_4)_2$.

Loss on drying. Anhydrous form, dry at 200° for 16 hours. Dodecahydrate, dry first at 50-55° for 1 hour, then at 200° for 16 hours.

Neutralizing value. Weigh accurately 500 mg of the anhydrous form into a 200-ml Erlenmeyer flask, add 30 ml of water and 4 drops of phenolphthalein T.S., and boil until the sample dissolves. Add 13.0 ml of 0.5 N sodium hydroxide, boil for a few seconds, and titrate with 0.5 N hydrochloric acid to the disappearance of the pink color, adding the acid dropwise and agitating vigorously after each addition. Calculate the neutralizing value, as parts of $NaHCO_3$ equivalent to 100 parts of the sample, by the formula $8.4V$, in which V is the volume, in ml, of 0.5 N sodium hydroxide consumed by the sample.

Ammonium salts. Heat 1 g with 10 ml of sodium hydroxide T.S. on a steam bath for 1 minute. The odor of ammonia is not perceptible.

Arsenic. A solution of 1 g in 35 ml of water meets the requirements of the Arsenic Test.

Fluoride. Determine as directed in the test for fluoride under Aluminum Ammonium Sulfate.

Heavy metals. Dissolve 1 g in 20 ml of water, add a few drops of diluted hydrochloric acid T.S., and evaporate to dryness in a porcelain dish. Treat the residue with 20 ml of water, and add 50 mg of hydroxylamine hydrochloride. Heat on a steam bath

for 10 minutes, cool, and dilute to 25 ml with water. This solution meets the requirements of the Heavy Metals Test, using 20 µg of lead ion (Pb) and 50 mg of hydroxylamine hydrochloride in the control (Solution A).

Lead. A solution of 1 g in 10 ml of water meets the requirements of the Lead Limit Test, using 10 µg of lead ion (Pb) in the control.

Selenium. A solution of 2 g in 40 ml of dilute hydrochloric acid (1 in 2) meets the requirements of the Selenium Limit Test.

Packaging and storage. Store in tight containers.

Functional use in foods. Buffer; neutralizing, firming agent.

VI. Description

A. General characteristics:

Sodium alum (as anhydrous or dodecahydrate) occurs as colorless crystals, or white granules or powder. It is odorless and has a saline, astringent taste.

B. Physical properties:

The anhydrous form is slowly soluble in water while the dodecahydrate is freely soluble. Both forms are insoluble in alcohol. (The dodecahydrate effloresces in air.) The dodecahydrate colorless cubic octahedral crystals have an index of refraction of 1.4388, a density of 1.675, and a melting point of 61°C.

C. Stability in containers:

Not available.

ALUMINUM AMMONIUM SULFATE

I. Nomenclature

A. Common names: Ammonium alum

B. Chemical names: Aluminum ammonium sulfate, dodecahydrate

Sulfuric acid, aluminum ammonium salt(2:1:1), dodecahydrate

C. Trade names: none

D. Chemical Abstracts Services Unique Registry Number:

7784250 (anhydrous)

7784261 (dodecahydrate)

II. Empirical Formula

$AlH_4NO_8S_2$ $(\cdot 12H_2O)$

III. Structural Formula

$AlNH_4(SO_4)_2$ $(\cdot 12H_2O)$

IV. Molecular Weight: 237.14 (anhydrous); 453.32 (dodecahydrate)

V. Specifications

A. The Food Chemicals Codex Second Edition (034) presents the following food grade specifications for ammonium alum:

1. Description

Large, colorless crystals, white granules, or a powder. It is odorless and has a sweetish, strongly astringent taste. One gram dissolves in 7 ml of water at 25° and in about 0.3 ml of boiling water. It is insoluble in alcohol, and is freely, but slowly soluble in glycerin. Its solutions are acid to litmus. A 1 in 20 solution gives positive tests for aluminum and for ammonium, and for sulfate.

2. Specifications

Assay. Not less than 99.5% of $AlNH_4(SO_4)_2 \cdot 12H_2O$.

Limits of impurities

Alkalies and alkaline earths. Passes test.

Arsenic (as As). Not more than 3 parts per million (0.0003%).

Fluoride. Not more than 30 parts per million (0.003%).

Heavy metals (as Pb). Not more than 20 parts per million (0.002%).

Lead. Not more than 10 parts per million (0.001%).

Selenium. Not more than 30 parts per million (0.003%).

3. Tests

Assay. Weigh accurately about 1 g, dissolve it in 50 ml of water, add 50.0 ml of 0.05 M disodium ethylenediaminetetraacetate, and boil gently for 5 minutes. Cool, and add in the order given and with continuous stirring 20 ml of pH 4.5 buffer solution (77.1 g of ammonium acetate and 57 ml of glacial acetic acid in 1000 ml of solution), 50 ml of alcohol, and 2 ml of dithizone. Titrate with 0.05 M zinc sulfate to a bright rose-pink color, and perform a blank determination. Each ml of 0.05 M disodium ethylenediaminetetraacetate is equivalent to 22.67 mg of $AlNH_4(SO_4)_2 \cdot 12H_2O$.

Alkalies and alkaline earths. Completely precipitate the aluminum from a boiling solution of 1 g of the sample in 100 ml of water by the addition of enough ammonia to render the solution distinctly alkaline to methyl red, and filter. Evaporate the filtrate to dryness, and ignite. The weight of the residue does not exceed 5 mg.

Arsenic. A solution of 1 g in 35 ml of water meets the requirements of the Arsenic Test.

Fluoride.

Lime suspension. Carefully shake about 56 g of low-fluorine calcium oxide (about 2 parts per million F) with 250 ml of water, and add 250 ml of 60% perchloric acid slowly and with stirring. Add a few glass beads, and boil to copious fumes of perchloric acid, then cool, add 200 ml of water, and boil again. Repeat the dilution and boiling once more, cool, dilute considerably, and filter through a fritted glass filter, if precipitated silicon dioxide appears. Pour the clear solution, with stirring, into 1000 ml of sodium hydroxide solution (1 in 10), allow the precipitate to settle, and siphon off the supernatant liquid. Remove the sodium salts from the precipitate by washing 5 times in large centrifuge bottles, shaking the mass thoroughly each time. Finally, shake the precipitate into a suspension and dilute to 2000 ml. Store in paraffin-lined bottles and shake well before use. (Note: 100 ml of this suspension should give no appreciable fluoride blank when evaporated, distilled, and titrated as directed in the Fluoride Limit Test.)

Procedure. Assemble the distilling apparatus as described in the Fluoride Limit Test, and add to the distilling flask 1.67 g of the sample, accurately weighed, and 25 ml of dilute sulfuric acid (1 in 2). Distil until the temperature reaches 160°, then maintain at 160° to 165° by adding water from the funnel, collecting 300 ml of distillate. Oxidize the distillate by the cautious addition of 2 or 3 ml of fluorine-free 30% hydrogen peroxide (to

remove sulfites), allow to stand for a few minutes, and evaporate in a platinum dish with an excess of Lime Suspension. Ignite briefly at 600°, then cool and wet the ash with about 10 ml of water. Cover the dish with a watch glass, and cautiously introduce under cover just sufficient 60% perchloric acid to dissolve the ash. Add the contents of the dish through the dropping funnel of a freshly prepared distilling apparatus (the distilling flask should contain a few glass beads), using a total of 20 ml of the perchloric acid for dissolving the ash and transferring the solution. Add 10 ml of water and a few drops of silver perchlorate solution (1 in 2) through the dropping funnel, and continue as directed in the Fluoride Limit Test, beginning with "Distil until the temperature reaches 135°..."

Heavy metals. Dissolve 1 g in 20 ml of water, add a few drops of diluted hydrochloric acid T.S., and evaporate to dryness in a porcelain dish. Treat the residue with 20 ml of water, and add 50 ml of hydroxylamine hydrochloride. Heat on a steam bath for 10 minutes, cool, and dilute to 25 ml with water. This solution meets the requirements of the Heavy Metals Test, using 20 µg of lead ion (Pb) and 50 mg of hydroxylamine hydrochloride in the control (Solution A).

Lead. A solution of 1 g in 10 ml of water meets the requirements of the Lead Limit Test, using 10 µg of lead ion (Pb) in the control.

Selenium. A solution of 2 g in 40 ml of dilute hydrochloric acid (1 in 2) meets the requirements of the Selenium Limit Test.

Packaging and storage. Store in well-closed containers.

Functional use in foods. Buffer; neutralizing agent.

VI. Description

A. General characteristics:

Ammonium alum exists as large, colorless crystals, white granules, or powder. It is odorless and has a styptic taste.

B. Physical properties:

Ammonium alum has a density of 1.65 and a melting point of 94.5°C. (Becomes anhydrous at about 250° and decomposes above 280°.) One gram dissolves in 7 ml of water (0.5 ml of boiling water), is freely soluble in glycerol and practically insoluble in alcohol.

C. Stability in containers:

Not available.

SODIUM ALUMINATE

I. Nomenclature

A. Common names: none available

B. Chemical names: Sodium aluminate

C. Trade names: none available

D. Chemical Abstracts Services Unique Registry Number: 7758170

II. Empirical Formula

$AlNaO_2$

III. Structural Formula

$NaAlO_2$

IV. Molecular Weight: 81.97

V. Specifications

Not available.

VI. Description

A. General characteristics:

Sodium aluminate occurs as a white granular mass.

B. Physical properties:

Sodium aluminate has a melting point of 1650°C; it is very soluble in water, insoluble in alcohol. The aqueous solution is strongly alkaline.

C. Stability in containers:

Not available.

ALUMINUM OLEATE

I. Nomenclature

A. Common names: none available

B. Chemical names: Aluminum oleate

Oleic acid, aluminum salt

C. Trade names: none available

D. Chemical Abstracts Services Unique Registry Number: 688379

II. Empirical Formula

$C_{54}H_{99}AlO_6$

III. Structural Formula

$(CH_3(CH_2)_7CH{=}CH(CH_2)_7COO)_3\ Al$

IV. Molecular Weight: 871.30

V. Specifications

Not available.

VI. Description

A. General characteristics:

Aluminum oleate occurs as a yellowish, viscid mass.

B. Physical properties:

The substance is practically insoluble in water, soluble in alcohol, benzene, ether, and oil of turpentine.

C. Stability in containers:

Not available.

ALUMINUM PALMITATE

I. Nomenclature

A. Common names: none available

B. Chemical names: Aluminum palmitate

Palmitic acid, aluminum salt

C. Trade names: none

D. Chemical Abstracts Services Unique Registry Number: 555351

II. Empirical Formula

$C_{48}H_{93}AlO_6$

III. Structural Formula

$[CH_3(CH_2)_{14}COO]_3Al$

IV. Molecular Weight: 793.25

V. Specifications

Not available.

VI. Description

A. General characteristics:

Aluminum palmitate is a white to yellow mass or powder.

B. Physical properties:

It is practically insoluble in water or alcohol; when fresh it is soluble in petroleum ether or turpentine.

C. Stability in containers:

Not available.

ALUMINUM HYDROXIDE

I. Nomenclature

A. Common names: Hydrated alumina
Aluminum trihydrate

B. Chemical names: Aluminum hydroxide

C. Trade names: Creamalin, Cremorin, Chefarox, Al-U-Creme, Alucol, Aludrox, Amphojel, Alkagel, Collumol, Aldrox, Hycolal, Hydrolum, Pepsamar, Fluagel, Gellumina, Vanogel

D. Chemical Abstracts Services Unique Registry Number: 21645512

II. Empirical Formula

AlH_3O_3

III. Structural Formula

$Al(OH)_3$

IV. Molecular Weight: 77.99

V. Specifications

A. The United States Pharmacopeia 18th Revision (189) presents the following specification for aluminum hydroxide (as gel, dried gel, and dried gel tablets):

1. Aluminum hydroxide gel is a suspension, each 100 g of which contains an equivalent of not less than 3.6 g and not more than 4.4 g of aluminum oxide (Al_2O_3), in the form of aluminum hydroxide and hydrated oxide. It may contain peppermint oil, glycerin, sorbitol, sucrose, saccharin, or other suitable flavors, and it may contain suitable antimicrobial agents in a total amount not exceeding 0.5%.

Description. White, viscous suspension, from which small amounts of clear liquid may separate on standing.

Identification. A solution in hydrochloric acid responds to the tests for aluminum.

Microbial limits. Its total aerobic microbial count does not exceed 100 per ml and it meets the requirements of the test for absence of Escherichia coli.

pH. Between 5.5 and 8.0, determined potentiometrically.

Acid-consuming capacity. Transfer about 1.5 ml of the well-shaken gel to a tared, glass stoppered, 125-ml flask, and weigh. Add 50.0 ml of 0.1 N hydrochloric acid, and shake the mixture continuously at 37° for 1 hour. Then add bromophenol blue, and titrate the excess acid with 0.1 N sodium hydroxide. The volume of 0.1 N acid consumed is not less than 12.5 ml and not more than 25.0 ml for each g of the gel.

Chloride. Transfer 10 g to a porcelain dish. Add 0.1 ml of potassium chromate and 25 ml of water. Stir, and add 0.1 N silver nitrate until a faint persistent pink color is obtained: not more than 8 ml of 0.1 N silver nitrate is required (0.28%).

Sulfate. Add 5 ml of diluted hydrochloric acid to 5 g of it, and heat to dissolve the sample. Cool, dilute with water to 250 ml, and filter if necessary: a 20-ml portion of the filtrate shows no more sulfate than corresponds to 0.2 ml of 0.02 N sulfuric acid (500 parts per million).

Arsenic. Dissolve 2.5 g in 20 ml of dilute sulfuric acid (1 in 5), and add 35 ml of water: the resulting solution meets the

requirements of the test, the addition of 20 ml of dilute sulfuric acid (1 in 5) specified under Procedure being omitted (0.6 part per million).

Heavy metals. Dissolve 5 g in 10 ml of diluted hydrochloric acid with the aid of heat, filter, if necessary, and dilute with water to 25 ml: the heavy metals limit is 5 parts per million.

Assay. Transfer about 25 g, accurately weighed, of aluminum hydroxide gel to a beaker, add 15 ml of hydrochloric acid, and heat gently until solution is complete. Cool, transfer to a 500-ml volumetric flask, dilute with water to volume, and mix. Pipet 20 ml of this solution into a 250-ml beaker, and add, in the order named and with continuous stirring, 25.0 ml of 0.05 M disodium ethylenediaminetetraacetate and 20 ml of acetic acid-ammonium acetate buffer T.S., then heat the solution near the boiling point for 5 minutes. Cool, and add 50 ml of alcohol and 2 ml of dithizone T.S. Titrate the solution with 0.05 M zinc sulfate until the color changes from green-violet to rose-pink. Perform a blank determination, substituting 20 ml of water for the sample, and make any necessary correction. Each ml of 0.05 M disodium ethylenediaminetetraacetate consumed is equivalent to 2.549 mg of Al_2O_3.

Packaging and storage. Preserve in tight containers, and avoid freezing.

Category: Antacid.

Usual dose: 15 ml four to six times a day.

Usual dose range: 5 to 30 ml up to twelve times daily.

2. Dried aluminum hydroxide gel yields not less than 50.0% of aluminum oxide (Al_2O_3).

Description. White, odorless, tasteless, amorphous powder.

Solubility. Insoluble in water and in alcohol; soluble in dilute mineral acids and in solutions of fixed alkali hydroxides.

Identification. Dissolve 500 mg in 10 ml of diluted hydrochloric acid, with gentle warming: the solution responds to the tests for aluminum.

Reaction. Agitate 1 g with 25 ml of water, and filter: the filtrate is neutral to litmus.

Acid-consuming capacity. Weigh accurately 200 to 250 mg, and transfer to a glass-stoppered, 250-ml flask. Add 100.0 ml of 0.1 N hydrochloric acid, and shake the mixture continuously at 37° for 1 hour. To 50.0 ml of the solution add bromphenol blue T.S., and titrate the excess acid with 0.1 N sodium hydroxide: the volume of 0.1 N acid consumed is not less than 250 ml for each g of dried aluminum hydroxide gel.

Chloride. Dissolve 1 g in 30 ml of diluted nitric acid, heat to boiling, add water to make 100 ml, and filter: 2 5-ml portion of the filtrate, diluted with an equal volume of water, shows no more chloride than corresponds to 0.6 ml of 0.02 N hydrochloric acid (0.85%).

Sulfate. Dissolve 330 mg in 15 ml of diluted hydrochloric acid, heat to boiling, add water to make 250 ml, and filter: a 25-ml portion of the filtrate shows no more sulfate than corresponds to 0.2 ml of 0.02 N sulfuric acid (0.6%).

Arsenic. Dissolve 2.5 g in 20 ml of dilute sulfuric acid (1 in 5), and dilute with water to 55 ml: the resulting solution meets the requirements of the test, the addition of 20 ml of dilute sulfuric acid (1 in 5) specified under Procedure being omitted (8 parts per million).

Heavy metals. Dissolve 400 mg in 10 ml of diluted hydrochloric acid with the aid of heat, filter if necessary, and dilute with water to 25 ml: the heavy metals limit is 60 parts per million.

Assay. Weigh accurately about 2 g of dried aluminum hydroxide gel, and dissolve in 15 ml of hydrochloric acid, with the aid of heat. Cool, transfer to a 500-ml volumetric flask, dilute with water to volume, and mix. Pipet 20 ml of this solution into a 250-ml beaker, and add, in the order named and with continuous stirring, 25.0 ml of 0.05 M disodium ethylenediaminetetraacetate and 20 ml of acetic acid-ammonium acetate buffer T.S., then heat the solution near the boiling point for 5 minutes. Cool, and add 50 ml of alcohol and 2 ml of dithizone T.S. Titrate the solution with 0.05 M zinc sulfate to a bright rose-pink color. Perform a blank determination, substituting 20 ml of water for the sample solution, and make any necessary correction. Each ml of 0.05 M disodium ethylenediaminetetraacetate is equivalent to 2.549 mg of Al_2O_3.

Packaging and storage. Preserve in tight containers.

Category: Antacid.

Usual dose: The equivalent of 300 mg of aluminum hydroxide four to six times a day.

Usual dose range: The equivalent of 300 mg to 5 g of aluminum hydroxide daily.

3. Dried aluminum hydroxide gel tablets contain an amount of aluminum oxide (Al_2O_3) equivalent to less than 62.0% and not more than 72.0% of the labeled amount of aluminum hydroxide.

Identification. Digest a portion of finely powdered tablets, equivalent to about 500 mg of dried aluminum hydroxide gel, with 10 ml of diluted hydrochloric acid with gentle warming, and filter: the filtrate responds to the tests for aluminum.

Disintegration. 30 minutes.

Weight variation. Meet the requirements for Tablets.

Assay. Weigh and finely powder not less than 20 dried aluminum hydroxide gel tablets. Weigh accurately a portion of the powder, equivalent to about 2 g of dried aluminum hydroxide gel, add 15 ml of hydrochloric acid, and heat until dissolved. Dilute with water to about 100 ml, mix, and filter quantitatively into a 500-ml volumetric flask, washing the filter with water. Proceed as directed in the Assay under Dried Aluminum Hydroxide Gel, beginning with "dilute with water to volume." Each ml of 0.05 M disodium ethylenediaminetetraacetate is equivalent to 3.900 mg of $Al(OH)_3$.

Packaging and storage. Preserve in well-closed containers.

Tablets available. Tablets usually available contain the following amounts of aluminum hydroxide: 300, 500, and 600 mg.

VI. Description

A. General characteristics:

Aluminum hydroxide is a white viscous suspension, or a white, bulky, amorphous powder.

B. Physical properties:

Aluminum hydroxide is practically insoluble in water, but is soluble in aqueous alkaline solutions or in HCl, H_2SO_4, and other strong acids in the presence of some water.

SODIUM PHOSPHOALUMINATE

No information could be found for sodium phosphoaluminate.

VII. Analytical Methods

There are no analytical methods in the literature for aluminum compounds and salts which differentiate between the various compounds and salts when present as a component of a food substance or biological material. Assuming that the researcher is interested primarily in the detection and analysis of the aluminum content of a substance, the following methods have been cited and are presented:

(1) "Aluminon" (ammonium tricarboxylic acid)

The colorimetric determination of aluminum is by formation of a red-colored lake; comparison of the color to standards is described by Roller (146). Under extremely controlled conditions of analysis, the test is reported to be sensitive to 0.0001 mg of aluminum. Roller found most desirable results when the solution was buffered to pH 6.3. No interference was found from 10 mg of Bu^{2+}, Ca^{2+}, Mg^{2+}, Zn^{2+}, Pb^{2+}; 0.1 mg of Co^{2+}, Cu^{2+}; 5 mg PO_4^{3-}. One mg of SiO, from crystallized silicate solution, gave a color equivalent to 0.001 mg of aluminum (probably due to an impurity of aluminum); 0.10 mg of Cr^{3+} at room temperature gave a color equivalent to 0.0005 mg Al (15 minutes), 0.001 mg Al (30 minutes), and 0.008 mg Al (18 hours). The Cr^{3+} interference can be overcome by raising the pH, though this will give a resultant decrease in the overall sensitivity of the test. Fe^{3+}, 0.010 mg, a major source of interference, gave a color equivalent to 0.005 mg aluminum. Chenery (028) describes a method for eliminating iron interference by forming a colorless iron complex with thioglycolic

acid. Table 1 shows the effect of iron on aluminum determinations when thioglycolic acid is used as an inhibitor.

Table 1 . Effect of Iron on Aluminum

Fe added (μg)	0	50	100	200	500
Al found (μg)	4.75	4.75	4.8	5.0	5.5
Difference (%)	0	0	1.0	5.3	15.8

Other factors cited as affecting results of the aluminon method are time, temperature, volume and concentration.

Olsen, Gee and McClendon (131) in examining the precision and accuracy of the aluminon method found the precision to be 1% (0.6% when refined techniques were employed) and an accuracy of the order 1 to 3%. When organic material is being assayed, it is necessary to ash the material prior to analysis. Exact details for a method of ashing prior to aluminon analysis are given by Cox et al. (036).

(2) Photometric Determination with 8-Hydroxyquinoline (8-HQ)

The direct colorimetric determination of aluminum with 8-hydroxyquinoline is dependent upon the solubility of the aluminum hydroxyquinolate in chloroform. The absorption maximum of the aluminum hydroxyquinolate is 3950 Å. Solutions of 8-hydroxyquinoline in chloroform showed negligible interference in the absorption range for the determination of aluminum. The extraction (carried out in a separating funnel) of the aluminum from an aqueous solution was found to give best results (at pH 9) when the solution of 8-hydroxyquinoline in chloroform was 1% (w/v) and the funnel shaken steadily for 6 minutes with occasional release of pressure.

Effective extractions of aluminum were obtained when the pH of the aqueous solution was in the 4.5-11.5 range except between 6.5 and 8. Best extractions were found at pH 5 or 9.

Tests for precision showed a standard deviation of $\pm$ 0.47 µg based on 14 determinations carried out on a sample known to contain 50 µg of aluminum. The accuracy is shown in the table below:

Aluminum taken, µg	Mean (3 determinations), µg	Apparent error, µg
9.7	9.1	- 0.6
19.4	19.9	+ 0.5
29.2	29.4	+ 0.2
38.9	39.7	+ 0.8
48.6	48.9	+ 0.3

Most interfering cations could be successfully eliminated by carefully controlling the pH at extraction, or by masking the interferences with an alkaline solution of potassium cyanide.

(3) The spectrographic method for determination of aluminum as presented by McCollum, Rask, and Becker (117) offers the following advantages over chemical methods: (1) greater sensitivity; (2) specificity, confusing other substances for aluminum is eliminated; (3) simplicity; (4) absence of all chemical reagents except atmospheric oxygen.

Materials tested are ashed in a silica dish over Bunsen flames. The spectrum of the resulting ash is excited in a 20,000-U condensed spark between vertical copper electrodes by placing 20-30 mg of the ash in a hollow of the lower electrode.

The secondary circuit contains a self-induction coil which serves to reduce the intensities of the lines due to air. The spectrum produced by McCollum et al. was dispersed and recorded on plates by means of a Hilger E 1 quartz prism spectrograph. The raie ultime are 3961.5 Å and 3944.0 Å. Absence of both lines indicates a concentration of less than 0.5 ppm; presence of line 3961.5 with absence of 3940.0 indicates concentrations of approximately 0.5 ppm to less than 1 ppm; and presence of both lines indicates concentrations of 1 ppm or more.

VIII. Occurrence

A. Plants

Aluminum, because it is so widely spread in the soil, occurs in the ash of all plants in quantities ranging from traces to considerable amounts (Campbell et al., 025). The mean content of aluminum in plants is estimated at 0.02% (200 ppm in dry matter) in herbaceous vegetables, with a mean value of 0.002% in the woody part of the plant (025). Certain plants accumulate aluminum to more than 1000 ppm in their dried leaves, but the only plant family in this group that is of direct interest to the consumer is the Theaceae. An analysis of tea leaves from Ceylon, Tanganyika, and Kenya showed from less than 100 ppm to more than 17,000 ppm of aluminum (dry basis), depending on the age of the leaves. In the commercial part of the plant (the flush), the Al content is most commonly around 150-220 ppm (025).

The aluminum content of various vegetables determined by Underhill and Peterman (187) is shown in Table 2. Generally speaking, in plants the outer leaves and coverings yield the greater concentrations of aluminum,

Table 2. Aluminum Content of Fresh Foods (187)

Material	Description	No. of sources	Aluminum content* fresh	average	Remarks
Beans, string	Varying degrees of ripeness	3	0.36 0.61 0.91	0.63	
Beets	Peeled	3	0.34 0.48 0.61	0.48	
Corn	Sweet Cut from cob	2	0.25 0.26	0.26	
Lettuce	Head	3	2.50 0.58 0.46	1.18	1st sample very young
Onions	Peeled	3	4.60 3.76 4.59	4.31	
Peas	Shelled	3	0.28 0.32 0.33	0.31	
Potatoes	Peeled	3	0.38 0.32 2.20	0.97	1st sample Florida 2nd sample Connecticut 3rd sample unknown
Apples	Variety unknown Duchess	1 1	0.015 0.08	0.047	
Cantaloupe	Edible portion	2	0.40 1.14	0.77	
Cherries	Pitted	2	3.18 3.80	3.49	Native sour California sweet
Oranges	Peeled	1	0.088	0.088	California seedless
Peaches	Peeled and stoned	2	0.93 0.83	0.88	Freestone Cling stone
Watermelon	Ripe heart	2	0.027 0.028	0.028	

*mg/100 g.

probably because of deposition of this element in external dust (025).

B. Animals

Herbivorous animals consume aluminum contained in plants, but most of this is apparently excreted with the feces (Hutchinson, 078). The best general value for mammalian tissue is 0.5 mg/kg, representing, according to Hutchinson, 1/40th the amount found in food.

The occurrence of aluminum in human tissue has been the subject of some controversy, which should not be surprising since amounts detected will vary according to diet, natural local occurrence of Al in dust and soil, and the analytical methods used. Kehoe et al. (087) find it present regularly in tissues in small amounts. They found a mean concentration of 0.013 mg/100 ml, which is lower than the values reported by other workers. Using their own method of spectrographic analysis, Kehoe et al. (087) reported the following values (in mg metal/100 g wet tissue) for normal human tissue: kidney, 0.042; heart, 0.056; brain, 0.004; liver, 0.160; spleen, 0.130; lung, 5.94; muscle, 0.015; long bone, 0.500; rib bone, 0.240; stomach, 0.73; intestines, 0.087. They hypothesize that the high concentration of Al found in lung tissue is a result of inhalation of dust carrying the metal. Campbell et al. (025) present in a review additional figures for Al found in human tissues, shown here in Table 3.

C. Synthetics

See the Biochemical Section VI, Consumer Exposure, for a discussion of aluminum in containers used for processing food.

D. Natural Inorganic Sources

Aluminum does not occur on the earth's surface in the metallic state, but in combination with oxygen, fluorine, silicon, and other elements

Table 3. Compilation of Data on the Occurrence of Aluminum in the Tissues of Man[a]

Tissue	Methods of analysis: Spectrographic	Rosolic acid	Alizarin	Not given
Whole blood (ppm)	0.13 ± 0.01[b] Nil-0.3 g 0.21-0.94[e], mode 0.54 0.1-0.5[f]	<2.0[c]	Trace - 2.10[d], av. 0.6	
Blood partition (ppm)	Plasma 0.24 ± 0.02[g] Cells 0.03 ± 0.003[h]	Plasma 0.15-0.5[i]		
Liver	0.160[j] 3.0[m]	0.05-0.14[k], av. 0.08	0.17-1.17[l]	
Kidneys	0.042[n] +(no figures given)[q]	0.02-0.34[o], av. 0.10	0.13-0.87[p]	
Gall bladder bile		0.01-0.17[r], av. 0.07		
Stomach	0.073[s]			
Small intestine	0.087[t]			
Lungs	5.94[u] +(no figures given)[v]			
Spleen	0.130[w]	0.03-0.13[x] av. 0.07		
Brain	0.004[y] Trace (ashed)[aa] 10 cerebral and cerebellar cortex (ashed)[bb]	0.07-0.45[z], av. 0.25		
Heart	0.056[cc] +(no figures given)[ee]	0.11-0.29[dd], av. 0.21		
Muscle	0.015[ff]			
Pancreas	+[gg]			
Testicles	+[hh]			
Long bone	0.500[ii]			
Rib bone	0.240[jj]			
Skin	Dry tissue 0.000000053-0.0000045[kk,ll]			

Tissue	Methods of analysis: Spectrographic	Rosolic acid	Alizarin	Not given
Hair	Dry tissue 0.0000000055-0.0000021[kk, mm]			
Nails	Dry tissue 0.000000045-0.000053[kk, nn]			
Whole organism				50-150 mg est. total[oo]

[a] Milligrams of Al per 100 g fresh tissue, except where indicated otherwise.
[b] Kehoe et al., J. Nutr. 19: 579-592 (1940).
[c] Myers and Mull, J. Biol. Chem. 78: 625-626 (1928).
[d] Underhill and Peterman, Am. J. Physiol. 90: 40-51 (1929).
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[i] Front and Kirsner, J. Lab. Clin. Med. 27: 1598-1605 (1942).
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[q] Dutoit and Zbinden, Comp. Rend. Akad. Sci. 190: 172-173 (1930).
[r] Myers and Mull, J. Biol. Chem. 78: 625-626 (1928).
[s] Kehoe et al., J. Nutr. 19: 579-592 (1940).
[t] Kehoe et al., J. Nutr. 19: 579-592 (1940).
[u] Kehoe et al., J. Nutr. 19: 579-592 (1940).
[v] Dutoit and Zbinden, Compt. Rend. Akad. Sci. 190: 172-173 (1930).
[w] Kehoe et al., J. Nutr. 19: 579-592 (1940).
[x] Myers and Mull, J. Biol. Chem. 78: 625-626 (1928).
[y] Kehoe et al., J. Nutr. 19: 579-592 (1940).
[z] Myers and Mull, J. Biol. Chem. 78: 625-626 (1928).

aa Voinar and Rusanov, Biokhimiya 14: 102-106 (1949); Chem. Abstr. 43: 6719 (1953).
bb Voinar, Biokhimiya 19: 29-33 (1953); Chem. Abstr. 47: 7625 (1953).
cc Kehoe et al., J. Nutr. 19: 579-592 (1940).
dd Myers and Mull, J. Biol. Chem. 78: 625-626 (1928).
ee Dutoit and Zbinden, Compt. Rend. Akad. Sci. 190: 172-173 (1930).
ff Kehoe et al., J. Nutr. 19: 579-592 (1940).
gg Dutoit and Zbinden, Compt. Rend. Akad. Sci. 190: 172-173 (1930).
hh Dutoit and Zbinden, Compt. Rend. Akad. Sci. 190: 172-173 (1930).
ii Kehoe et al., J. Nutr. 19: 579-592 (1940).
jj Kehoe et al., J. Nutr. 19: 579-592 (1940).
kk There is no good explanation for these tissues containing so little aluminum.
ll Goldblum et al., J. Invest. Dermat. 20: 13-18 (1953).
mm Goldblum et al., J. Invest. Dermat. 20: 13-18 (1953).
nn Goldblum et al., J. Invest. Dermat. 20: 13-18 (1953).
oo Heupke, Munch. Med. Wchnschr. 92: 351-358 (1950).

(Campbell et al., 025). Nevertheless, it is the most abundant metallic element, constituting 8% of the earth's crust (025). It is concentrated in the lithosphere, but is also found in the hydrosphere and atmosphere, and is third in order of abundance (7.51%) after oxygen and silicon (025). It occurs in undecomposed rock fragments, in secondary aluminosilicate clays, as a solid or hydrosol hydrated oxides and phosphates, and in some cases in ionic form (025). Bauxite is the most important ore for production of purified alumina, cryolite the second most important, and alum-hydrated aluminum sulfate- the third. The crystalline double salts of the latter, -ammonium aluminum sulfate and potassium aluminum sulfate- may occur as minerals in nature, but the information on this subject is not clear (025).

Aluminum is washed into soils from decomposed rock fragments. Campbell et al. (025) assembled data on the occurrence of aluminum in some soils in the United Stated, reproduced here in Table 4. From the soil, the aluminum is carried into water, but the concentration is negligible because aluminum compounds in water are precipitated out of solution or adsorbed on sediments, leaving only traces (025). Sea water has a relatively low concentration and public water, if properly treated, should not contain significant amounts of Al (see Table 7 in Campbell et al., 025 for amounts in drinking water supplies).

The presence of aluminum in airborne particulate matter is to be expected as a consequence of its occurrence in soil. However, since the concentration will vary with meterological conditions, vehicular traffic, location of sampling site and type of sample, any data given here would not necessarily be representative. It should be noted that the aluminum content of air is generally rather high in coal-burning areas (025).

Table 4. Occurrence of Aluminum in Some Soils of the United States

State	Soil	Parts per million
Alabama	Top	46,037
	Sub	67,725
Connecticut	Top	5,344-44,233
	Sub	8,730-36,614
District of Columbia	Top	32,963-43,069
	Sub	19,048
Florida	Top	11-135,079
	Sub	85-26,349
Georgia	Top	1,296-246,984
	Sub	5,767-238,730
Maine	Top	65,767-68,889
	Sub	72,910-77,407
Maryland	Top	20,370-146,190
	Sub	9,894-154,815
Massachusetts	Top	11,058-58,677
	Sub	54,603-58,995
Missouri	Top	51,164
	Sub	71,111
New Hampshire	Top	30,899-98,624
	Sub	37,302-70,053
New Jersey	Top	159-42,116
	Sub	2,645-97,196
New York	Top	635-55,502
	Sub	741-83,915
North Carolina	Top	10,952-90,529
	Sub	46,667-145,926
Pennsylvania	Top	19,550-60,264
	Sub	29,312-78,307
Rhode Island	Top	14,524-29,069
South Carolina	Top	259-108,465
	Sub	3,201-140,401
Virginia	Top	7,249-132,645
	Sub	13,328-265,449
West Virginia	Top	22,751-42,963
	Sub	28,413-74,973
Wisconsin	Top	48,148
	Sub	50,212

BIOLOGICAL DATA

I. Acute Toxicity

A. Mice

1. Hart and Adamson (069) injected 10 CDF, male mice (age not given) intraperitoneally daily for 10 consecutive days with aluminum nitrate [$Al(NO_3)_3 \cdot 9H_2O$] dissolved in 0.9% saline and given in a volume of 0.01 ml/g BW. The number of deaths occurring within a 30-day observation period was noted.

2. Ondreicka et al. (134) determined the oral (by probe) LD_{50} values for aluminum chloride and aluminum sulfate with male white mice (average weight 200 g) from the Slovakian Academy of Science, Dobra-Voda. The authors state that the values found (see Acute Toxicity Table 5) place these aluminum salts in the category of compounds with low acute toxicities according to certain previously established criteria.

B. Rats

1. Underhill et al. (185) injected 2 rats (strain, age, sex not given) subcutaneously in the thigh with a 20% solution of aluminum chloride in distilled water (for dosages see Table 6). The rat receiving 7 g/kg died on the second day and the one receiving 8 g/kg on the third day following injection. The first symptom observed was loss of appetite followed by inactivity and depressed behavior (the rats lay quietly in cages, showing no interest in surroundings and when forced to move were slow and clumsy).

Gross examination showed marked congestion in all the viscera, particularly the mesenteric vessels. The stomach was distended, and the somewhat swollen mucosa contained a few tiny hemorrhages.

Table 5 . Acute Toxicity

Substance	Animal (species)	Sex & No. (M or F)	Route (p.o., i.v., s.c., i.p., i.m., other)	Dosage (mg/kg body wt.)	Measurement (LD_{50}, ED_{50} or other)	Ref. Bibliogr. No.
Aluminum nitrate [$Al(NO_3)_3 \cdot 9H_2O$]	CDF_1 mice	M, 10	i.p. (10 consec. days)	213	LD_{10}	Hart, M.M., and Adamson, R.H. (69)
Aluminum nitrate	CDF_1 mice	M, 10	i.p. (10 consec. days)	320 (282-374)	LD_{50}	Hart, M.M., and Adamson, R.H. (69)
Aluminum nitrate	Sprague-Dawley rats	F, 8	i.p. (10 consec. days)	240	LD_{10}	Hart, M.M., and Adamson, R.H. (69)
Aluminum nitrate	Sprague-Dawley rats	F, 8	i.p. (10 consec. days)	327 (283-378)	LD_{50}	Hart, M.M., and Adamson, R.H. (69)
Aluminum chloride	Mice	M, no. not given	p.o.	770 $\pm$ 120 mg Al/kg	LD_{50}	Ondreicka, R., et al. (134)
Aluminum chloride	Rats	M, 5	p.o.	1100 mg Al/kg	LD_{100}	Berlyne, G.M., et al. (014)
Aluminum chloride	Rats	not given	s.c.	7000-8000	LD_{100}	Underhill, F.P., et al. (185)
Aluminum chloride	Guinea pigs	not given	s.c.	5000-7000	LD_{100}	Underhill, F.P., et al. (185)
Aluminum chloride	Rabbits	not given	s.c.	7000-8000	LD_{100}	Underhill, F.P., et al. (185)

Table 5 (continued)

Substance	Animal (species)	Sex & No. (M or F)	Route (p.o., i.v., s.c., i.p., i.m., other)	Dosage (mg/kg body wt.)	Measurement (LD_{50}, ED_{50} or other)	Ref. Bibliogr. No.
Aluminum sulfate	Mice	M, no. not given	p.o.	980 ± 90 mg Al/kg	LD_{50}	Ondreicka, R., et al. (134)
Aluminum sulfate	Rats	M, 10	p.o.	1100 mg Al/kg	LD_{100}	Berlyne, G.M., et al. (014)
Aluminum sulfate	Rabbits	not given	s.c.	7000-8000	LD_{100}	Underhill, F.P., et al. (185)
Aluminum hydroxide	Rats	M, 5	i.p.	150 mg Al/kg/day	LD_{100}	Berlyne, G.M., et al. (185)

Pathological study showed that the liver and kidney were the most extensively damaged organs. Central necrosis and fatty infiltration were seen in the liver. The convoluted tubule and glomerular tufts were the damaged areas noted in the kidney.

2. Hart and Adamson (069) injected 8 female Sprague-Dawley rats (age not given) intraperitoneally daily for ten consecutive days with aluminum nitrate [$Al(NO_3)_3 \cdot 9H_2O$] dissolved in 0.9% saline and given in a volume of 0.01 ml/g BW. The number of deaths occurring within a 30-day observation period was noted.

3. Berlyne et al. (014) gave white adult male rats (Weizmann Institute strain, 200 g) aluminum chloride or aluminum sulfate (1% or 2%) in their drinking water and determined that the minimum fatal dose was 1100 mg Al/kg BW. When aluminum hydroxide was given intraperitoneally (150 mg Al/kg/day) to 5 rats, all developed periorbital bleeding and died.

C. Guinea Pigs

Underhill et al. (185) injected 5 guinea pigs (age and sex not given) subcutaneously in the thigh with a 20% solution of aluminum chloride in distilled water (for dosage schedule as related to duration of life see Table 6). The lethal dose was 5-7 g/kg BW when the initial dose was 3 g followed by 1 g on alternating days. This dose produced death in 3-7 days. The symptoms and organ damage were similar to those observed in the rats (see Acute Toxicity Section B, 1).

D. Rabbits

1. Underhill et al. (185) injected 2 rabbits (age and sex not given) subcutaneously in the thigh with a 20% solution of aluminum chloride in distilled water and 3 other rabbits similarly with a 20% solution of

Table 6 .

Dosages and Duration of Life of Animals Receiving $AlCl_3$ and $Al_2(SO_4)_3$ Subcutaneously[a]

Animal	Number	Initial dose (g)		Subsequent dose	Total (g)	Death
		$AlCl_3$	$Al_2(SO_4)_3$			
Rat	1	7		None	7	3rd day
	2	8		None	8	2nd day
Guinea pig	1	3		1 g alternate days	5	7th day
	2	3		1 g alternate days	6	5th day
	3	3		1 g alternate days	5	8th day
	4	5		1 g alternate days	7	4th day
	5	3		1 g alternate days	7	6th day
Rabbit	1	5		1 g 8th and 10th day	7	10th day
	2	4		1 g 3rd, 5th, and 8th day	7	8th day
	3		4	1 g 3rd, 5th, 7th and 9th day	8	10th day
	4		4	1 g 3rd, 5th and 7th day	7	8th day
	5		4	1 g 3rd, 5th, 7th and 10th day	8	11th day

[a]Doses as grams of salt per kilo body weight.

aluminum sulfate in distilled water (for dosage schedule as related to duration of life see Table 6). The lethal dose of either the chloride or sulfate is 7-8 g/kg BW when the initial dose is 4-5 g followed by 1 g on alternating days. This dose produced death in 7-11 days (see Table 6). The symptoms and organ damage to the rabbits were similar to those observed in the rats (see Section I, B, 1).

II. Short-Term Studies

Introduction

The effects of aluminum salts and aluminum baking powders on digestion and health have been studied by numerous experimenters. The results range from serious detrimental effects on growth, reproduction, and the gastrointestinal tract to no observed appreciable injurious effect. These somewhat conflicting studies are summarized below.

A. Mice

1. Schaeffer and co-workers (156) showed that mice developed inflammatory lesions in the gastrointestinal mucous membrane after prolonged ingestion of special breads leavened with alum or alum-phosphate baking powder. They performed two series of experiments with mice. In the first series, mice (strain and age not given) were divided into three groups which were fed a specially prepared bread diet for 4 months as follows:

(1) Group I, controls (20 couples), special bread made with yeast.

(2) Group II (20 couples), the same composition bread prepared with aluminum phosphate (2.07 g Al/1000 g bread).

(3) Group III (20 couples), the same composition bread leavened with alum baking powder (4.1 g Al/1000 g bread).

Serious lesions of the digestive tract were found in all animals from Groups II and III. No lesions were found in Group I mice. The lesions varied in extensiveness and were described as attacking both the epithelial layer and vascular tissue of the papillary axis. They resembled those seen in human pathology.

The authors attribute these lesions to the formation of soluble aluminum salts in the digestive tract. In support, they note that the lesions are not produced when an excess of physiological saline mixture is introduced into the food. They postulate that the lesion-preventing action of the saline is owed to the reaction of the hydrochloric acid with the other cations present, thus hindering the formation of aluminum chloride. They note that this protective reaction does not take place when humans eat baked goods containing alum baking powders.

Reproduction studies for these same three groups showed:

(1) Group I (bread with yeast); 300 offspring; normal ovaries.

(2) Group II (bread with aluminum phosphate): 193 offspring; the ovaries of all the female mice had a greater than normal proportion of atretic follicles.

(3) Group III (bread leavened with alum); 71 offspring; the most distinct ovarian lesions were found in all the female mice in this group.

In the second series of experiments with mice (strain and age not given), four groups of ten couples each were fed the following diet for 4 months.

(1) Group I, bread with yeast plus 4% physiological saline mixture (Osborne and Mendel salts mixture).

(2) Group II, bread with yeast plus 13% of the same saline mixture as Group I.

(3) Group III, bread with alum-phosphate baking powder (4.4% Al plus 4% of the same saline mixture as the first two groups).

(4) Group IV, bread with alum-phosphate baking powder (1.3% Al), no added saline mixture.

The following was observed after 4 months:

(1) Group I; 428 offspring (46 litters) with 6% mortality during the first week. Females had normal ovaries.

(2) Group II; 310 offspring (45 litters) with 10% mortality during the first week. Females had normal ovaries.

(3) Group III; 192 offspring (35 litters) with 23% death rate during the first week. The ovaries were reduced in size by almost half that of the controls and showed characteristic lesions. The absence of normal follicles as well as numerous atretic follicles was observed.

(4) Group IV; 244 offspring (42 litters) with 10% mortality during the first week. The ovaries showed characteristics similar to those found in Group III.

In male mice ingesting aluminum, there was no abnormality observed in the testes or in spermatogenesis.

The authors show concern that in the United States aluminum salts are permitted in baking powder when in most European countries the use of aluminum salts in food is considered dangerous.

The authors have shown that, contrary to the opinion that aluminum phosphate is insoluble and will not be absorbed by the digestive tract,

it is in fact soluble in the hydrochloric acid in the gastric juice. In the gastric juice the insoluble aluminum phosphate becomes soluble aluminum chloride. The dilute solutions of aluminum chloride formed then come in contact with the gastric, pyloric, and duodenal mucosa. For this reason the authors urged that alum leavens be prohibited.

2. Ondreicka et al. (134) studied the effects of low doses of aluminum chloride on the growth rate of three generations of mice. Ten white mice (Dobra Voda strain) were given aluminum chloride in their drinking water (average 19.3 mg Al/kg/day) for 180-390 days. Weights of animals, number of litters, and number of offspring were recorded, along with regular checks on the daily food and water intake. There were 10 controls. Figure 1 shows the weight changes for three generations. The 4-week old weanlings were treated in the same way as their parents. After sacrifice, the liver, spleen, kidneys, and blood were examined.

The observations made were:

(1) Treated and control mice showed no significant differences in numbers of litters or offspring.

(2) A marked growth retardation which was related to aluminum intake began to show up in the litters subsequent to the first litter (see Fig. 1).

(3) There was no significant difference in the erythrocyte count or hemoglobin level between the first and last generations and the controls.

(4) No pathological changes were found in the liver, spleen, and kidneys.

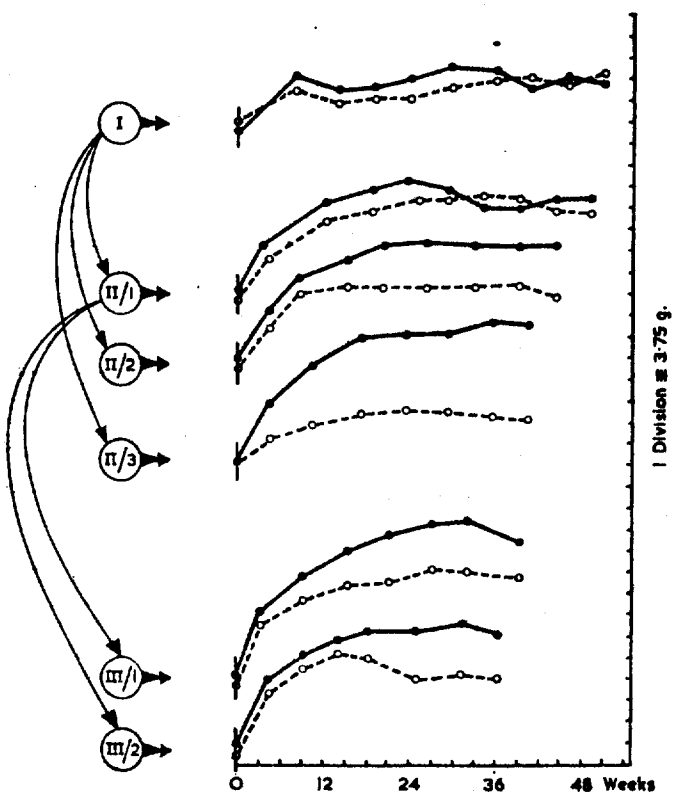

Fig. 1 . Weight increases of normal (full lines) and of aluminum trichloride treated (dotted lines) white mice: I, original generation; II/1,2,3, first, second, and third litter of the second generation; III/1,2, first and second litter of the third generation.

The authors conclude that retardation of growth was caused by relatively small doses of an aluminum compound, which is contrary to earlier studies maintaining that aluminum exerted no toxic effect at such doses. They point out, however, that since neither the morphology of various tissues nor the blood count showed any significant abnormalities, it was necessary to seek other, more sensitive indicators of metabolic disturbances resulting from chronic ingestion of small amounts of aluminum compounds.

B. Rats

1. Lyman and Scott (111) studied the rate of growth, longevity, reproduction, kidney function (as indicated by nonprotein nitrogen in the blood), and kidney pathology in young albino rats fed diets containing relatively small to large doses of either sodium aluminum sulfate-calcium acid phosphate baking powder (S.A.S. powder) or cream of tartar baking powder. Both baking powders used were commercial brands, Calumet (S.A.S. phosphate type) and Royal (tartrate type) purchased locally by the experimenters. Before the baking powders were mixed with the control diet, both were prepared by first moistening with water, then heating in an oven (1 hour at 102°) followed by air-drying and finally grinding to a powder (40 mesh). The various diets were fed ad libitum to seven groups (24 animals/group) of young albino rats (both sexes, 30-50 g). Table 7 shows the seven different diets along with the rates of growth, maximum size, and number of deaths occurring within 5 months of the onset of the experiment. The sexes were separated because rate of growth varied slightly with sex. It was found that the average time for weight gain was:

(1) 47 days for the 8 control males to gain 100 g.

(2) 45.1 days for the 26 aluminum-fed males to gain the same amount.

Table 7.

Diet	Av. time to gain from 60 to 160 g, days	Maximum variations, days	Av. Max. wt. of rats living 5 months or longer	No. dying under 5 months after starting diet	Total no. of rats	No. animals used to calculate rate of gain
			Males			
Control	47	35 to 56	260	2	13	8
Control + S.A.S. 1 to 223	47	42 to 56	275	2	10	8
Control + S.A.S. 1 to 56	42	28 to 59	263	1	14	11
Control + S.A.S. 1 to 669	46.5	28 to 59	263	0	8	7
Control + tartrate 1 to 116	47.5	38 to 59	261	3	10	6
Control + tartarte 1 to 29	44	28 to 59	277	5	14	8
Control + tartrate 1 to 340	57	49 to 70	284	3	8	3

Table 7 (continued)

Diet	Av. time to gain from 60 to 160 g, days	Maximum variations, days	Av. Max. wt. of rats living 5 months or longer	No. dying under 5 months after starting diet	Total no. of rats	No. animals used to calculate rate of gain
	To gain from 50 to 130 g		Females			
Control	40	31 to 56	193	2	9	4
Control + S.A.S. 1 to 223	37	33 to 45	180	7	14	6
Control + S.A.S. 1 to 56	39	28 to 59	207	2	17	9
Control + S.A.S. 1 to 669	37	16 to 66	190	3	13	5
Control + tartrate 1 to 116	40	31 to 52	212	4	14	7
Control + tartrate 1 to 29	52	31 to 63	197	4	14	7
Control + tartrate 1 to 340	54	38 to 84	192	2	10	5

(3) 40 days for 4 control females to gain 80 g.

(4) 37.6 days for 20 aluminum-fed females to gain the same amount. The amount of aluminum ingested did not alter the increase in growth (see Scott and Helz, 161). The maximum longevity on all the diets could not be determined owing to an epidemic of lung infection. The average longevity on the various diets was:

Diet	Average length of life in months	
	Male	Female
Control diet	10	7
S.A.S. acid phosphate	10	8.7
Tartrate	6.7	10
Highest S.A.S. (control diet + S.A.S., 1 to 56)	9	11
Highest tartrate (control diet + tartrate, 1 to 29)	9	10

On the assumption that injury to the tissues of the body would cause a derangement of kidney function and thus a rise in the blood nonprotein nitrogen, the authors determined this factor by the Folin-Wu method. Their findings are shown on Table 8.

Macroscopic and microscopic studies were also made of the kidneys of one animal from each feeding group (46 in all) killed at intervals from 45 days (after onset of experiment) to 21 months (end of experiment).

In a reproductive study, six generations were raised on rations containing approximately 2% S.A.S. baking powder residue for the first and second generations and 4% for the succeeding generations. Table 9 shows the reproduction record for two pairs of rats (third generation) fed with a diet of 1 part to 28 (S.A.S. baking powder to control diet) from weaning onward. No reproduction records could be made for the rats fed tartrate baking powder owing to loss of the second generation from infectious disease.

Table 8. Nonprotein Nitrogen in Rats' Blood[a]

Diet	Sept 6, 1927	Nov. 4, 1927	Jan. 4, 1928	Mar. 4, 1928	June 4, 1928	Average
Control	44	43	37.2		36	40
Control + 1 g S.A.S. to 223 g food	44	40	40	48	38	42
Control + 1 g S.A.S. to 56 g food	23	33	40	43	32	34
Control + 1 g S.A.S. to 669 g food	30	41	29	48	36	37
Control + 1 g tartrate to 116 g food	44	49	29	39	43	41
Control + 1 g tartrate to 29 g food	19	35	30	50	38	34
Control + 1 g tartrate to 340 g food	38	41	38	40	41	40

[a]In mg/100 ml.

Table 9. Reproduction on S.A.S.-Calcium acid phosphate diet

Pair no.	Date: Birth of young	No. young	Av. birth, wt. grams	Weaning age, days	Av. weaning, wt. grams	Total litter at weaning, grams	No. weaned
1	March 2, 1929.....	11	5	24	29	262	9
	April 6, 1929.....	12	5.8	20	38	188	5
	May 20, 1929.....	13	4.5	20	28.5	171	6
	July 5, 1929.....	9	5.4	21	30.0	242	8
	August 2, 1929.....	11	5.2	20	25.6	282	11
	September 4, 1929.....	7	5.3	21	39.3	275	7
	October 1, 1929.....	9	5.6	21	39.5	237	6
	October 25, 1929.....	7	5.5	All died			
2	March 5, 1929.....	12	5.6	21	30.0	150	5
	April 27, 1929.....	11	5.8	20	35.0	278	8
	June 10, 1929.....	13	5.3	21	26.0	340	13
	July 22, 1929.....	4	6.4	20	33	231	7
	August 16, 1929.....	7	5.4	21	33	198	6
	September 17, 1929.....	6	6.0	21	41	246	6

The authors' observations from these various studies were:

(1) There was no appreciable effect on the rate of growth, maximum adult size, longevity, reproduction, and nonprotein nitrogen in the blood, when white rats ingested rations containing S.A.S. phosphate baking powder in amounts up to approximately 2% of the diet.

(2) The same observations were made for the ingestion of tartrate baking powder by white rats in varying amounts up to 4% of the diet.

(3) Six generations were raised on rations containing 2% S.A.S. baking powder for the first and second generations and 4% for the succeeding generations. No difference was noted in rate of reproduction, number of young successfully weaned, and rate of growth of young between the experimental animals and the controls.

(4) Neither gross nor microscopic lesions were found in the kidneys of the rats on the various diets with baking powder which distinguished them from the kidneys of the controls.

The authors conclude that neither of the baking powders containing aluminum was injurious to the animals.

2. Scott and Helz (161) studied the effect of ingesting aluminum chloride on the growth, hemoglobin and blood count, liver iron content, and organ pathology of rats. They fed 5 white rats (3-4 weeks old, av. 40 g BW) a diet mash plus 3.6% aluminum chloride ($AlCl_3 \cdot 6H_2O$) for 57 days. The controls (6) received only the mash. At the end of the 57-day period the aluminum-fed animals had gained more weight than the

controls (204 g average for aluminum group; 191 g average for controls). The blood counts and hemoglobin for all the aluminum-fed animals and three of the controls taken at the end of 57 days are shown on Table 10.

After sacrifice, the iron content of the livers of the controls (4.81 mg iron/100 g liver) and of the test animals (4.96 mg iron/100 mg liver) was found to be normal.

The following organs were examined both macroscopically and microscopically: heart, both lungs, spleen, liver, both kidneys, both adrenals, cardiac and pyloric portions of the stomach, both testes and epididymes, ovaries, pancreas, duodenum, jejunum, cecum, and colon. No pathological changes were observed.

The authors concluded that the protracted ingestion of a 3.6% concentration of aluminum chloride had no deleterious effect on the growth, reproduction, or blood picture of the organs examined in the white rat.

3. Jones (082) produced a marked rickets accompanied by low serum phosphate in 22 rats when 4% basic aluminum acetate or 5% aluminum sulfate [$Al_2(SO_4)_3 \cdot 18H_2O$] was added to a stock diet. No details are given for this experimental study with aluminum salts. In another series of experiments when 22 young rats were fed either aluminum sulfate or aluminum acetate in addition to a stock diet for about 3 weeks (thereafter the metal was eliminated from the diet), a few (number not given) were observed in tetany accompanied by a marked rise in serum phosphorus and a drop in serum calcium.

Adult female rats (6) were fed a stock diet to which 5% aluminum sulfate [$Al_2(SO_4)_3 \cdot 18H_2O$] was added for 3 months (6 female controls received the same diet without aluminum). After sacrifice the following were determined:

Table 10.

Rat no.	Dare HB percent	Red cells	White cells
		Test	
1	80	7,650,000	12,100
2	80	7,050,000	18,400
3	110	9,980,000	21,500
4	95	9,000,000	23,000
5	90	9,800,000	16,500
		Control	
6	95	8,600,000	9,800
7	100	10,000,000	12,900
8	110	9,400,000	13,200

Averages for	Aluminum-fed	Controls
Serum calcium	11.8 mg %	12.6 mg %
Serum phosphate	2.3 mg %	4.7 mg %
Femur ash-calcium	146 mg %	223 mg %
Femur ash-phosphorus	53.3 mg %	62.0 mg %

In the aluminum-fed animals there was a marked decrease in serum phosphate and no rise in serum calcium. Nearly one-third of the mineral content of the skeleton was lost.

4. McCollum et al. (117) described the effects of adding aluminum chloride and alum baking powder (sodium aluminum sulfate, calcium acid phosphate, S.A.S. powder) to the diets of young growing rats. Two separate and independent feeding experiments were performed over 8 months. The rats used, weighing 45-55 g each, were from the researchers' breeding stock. Ten rats were fed the control diet with 0.6% aluminum chloride added ($Al_2Cl_6 \cdot 12H_2O$), representing 0.067% Al. Six rats were fed the control diet plus 3% S.A.S. powder (0.063% Al) and ten rats were fed the control diet only. Figures 2, 3, and 4 show the growth and reproduction frequencies for the three groups. The original paper also shows photographs of typical rats from each group. At the termination of the experiment (8 months), the livers, kidneys, spleens, ovaries, and testes of four of the first-generation test rats were studied.

The authors concluded that there is no noticeable deleterious action on the growth, reproduction, and general well-being as evidenced by both external appearance and autopsy, when rats are fed these aluminum compounds in their diets in concentrations as high as 60 ppm.

5. Berlyne et al. (014) recently (1972) showed that low doses of aluminum salts can be harmful to rats, particularly those with renal damage.

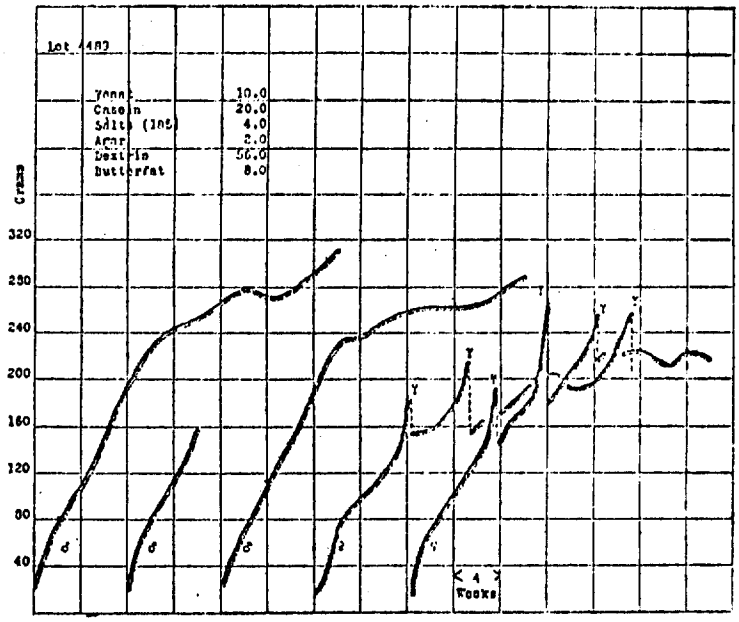

Fig. 2 . Growth records of control group of rats on aluminum-free diet.

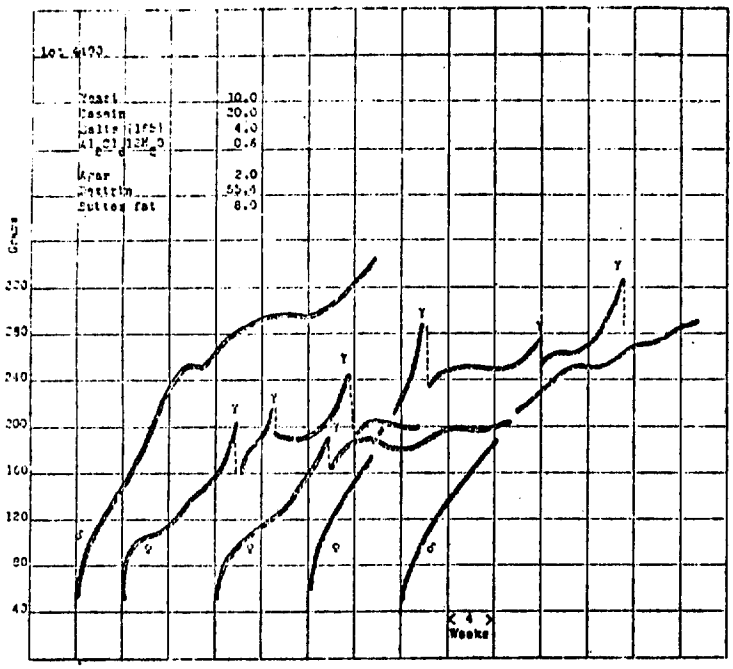

Fig. 3 . Growth records of group of rats fed aluminum chloride-containing diet.

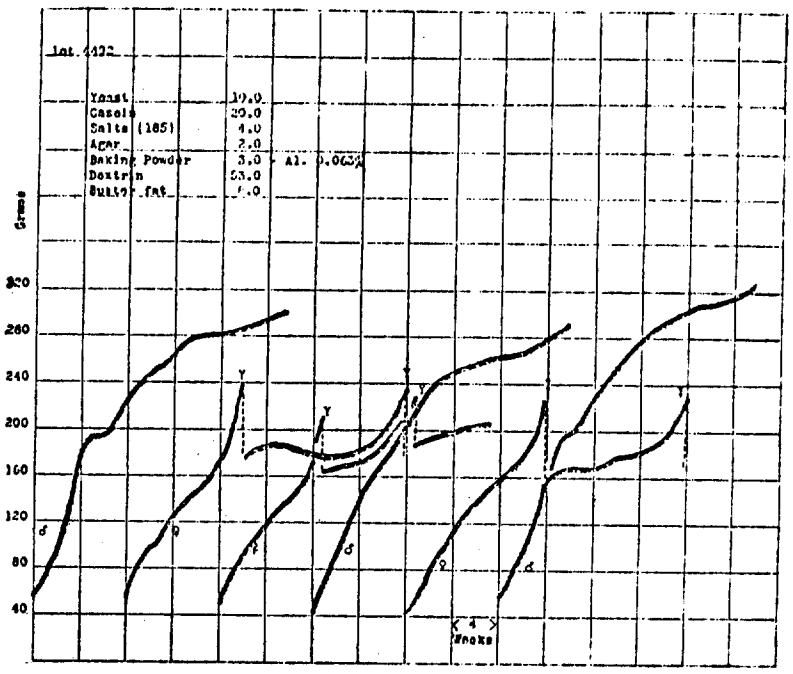

Fig. 4 . Growth records of group of rats fed aluminum in the form of sodium aluminum sulfate baking powder.

Table 11 shows the doses and test procedures used when aluminum sulfate or chloride (1 or 2%) and aluminum hydroxide (3 ml/day, equivalent to 90 or 150 mg Al/kg BW/day) were administered by various routes (drinking water, gavage, subcutaneous, and intraperitoneal) to littermate white adult male rats (Weizmann Institute strain, 200 g) divided into control and test groups (number in each group shown on Table 11). Some of the animals were five-sixths nephrectomized (total nephrectomy on one side and a two-thirds nephrectomy on the other).

The observations clinically were:

(1) All nephrectomized animals given 1% aluminum salts died within 8 days and all given 2% salts died within 3 days.

(2) These test rats showed: lethargy, failure to thrive, bleeding around the eyes, reduced spontaneous movement, and reduced water intake toward end of life (5-15 ml/day over 4 days for rats on 2% doses; 16-21 ml/day over 8 days for rats on 1% doses). All other animals drank 26-30 ml/day (intraperitoneal, subcutaneous and controls).

(3) Bone, brain, liver, heart, and muscle contained massive deposits of aluminum. (See tables in original paper.)

(4) Plasma aluminum levels in the uremic rats, normally 0.2 mg/liter, were raised between 0.5 and 40 mg/liter, depending on route of administration of the aluminum salt.

The authors note that this plasma level in uremic rats corresponds to that of human patients in renal failure who are administered various aluminum compounds therapeutically. They warn, therefore, that since the toxic dose for the uremic rat (90 mg/kg BW) closely corresponds to the

Table 11.

Design of In Vivo Toxicity,
Including Route of Administration and Mortality

Aluminum salt	Route	Dose (mg Al/ kg/day)	No. of rats	No. with peri-orbital bleeding	No. of deaths
5/6 nephrectomised					
2% $Al_2(SO_4)_3$	Drinking-water	375	10	10	10
1% $AlCl_3$	Drinking-water	180	5	3	5
1% $Al_2(SO_4)_3$	Drinking-water	300	5	4	5
$Al(OH)_3$	Gavage	150	5	0	1
$Al(OH)_3$	s.c.	150	5	0	0
$Al(OH)_3$	i.p.	150	10	10	10
Control					
Water	Drinking-water	Nil	10	0	0
2% $Al_2(SO_4)_3$	Drinking water	350	5	3	0
1% $Al_2(SO_4)_3$	Drinking water	200	5	0	0
1% $AlCl_3$	Drinking water	250	5	0	0
$Al(OH)_3$	Gavage	150	5	0	0
$Al(OH)_3$	s.c.	150	8	0	0
$Al(OH)_3$	i.p.	150	5	5	5

therapeutic dose (45-60 g/day) given to uremic humans, until further work is done patients with renal impairment should not be given aluminum salts.

Furthermore, they have found higher aluminum levels in the bones of uremic persons who have not taken aluminum salts than in nonuremic persons. They also feel that aluminum salts are implicated in a metatarsal type of osteoporosis seen in Newcastle, England where the aluminum content of bone is particularly high. The authors cite as evidence for their belief that aluminum may damage bone formation, a study which showed that 1 mg of aluminum can initiate hydroxyapatite precipitation.

Their conclusion is that in the light of recent studies, the widespread use of aluminum salts in man should be suspended until further studies are done.

6. Thurston et al. (179) who also studied the toxicity of aluminum in renal failure came to smoewhat different conclusions from Berlyne et al. (014). They administered the following diets to groups of six weanling rats (sex and strain not given) of the same mean weight (not given): (a) whole meal diet, (b) whole meal diet with 3.2 g/kg aluminum hydroxide added, (c) diet with added aluminum hydroxide plus 10 g/kg disodium hydrogen phosphate, (d) diet (b) given to three-quarter partially nephrectomized animals with a contralateral nephrectomy. The animals were weighed weekly and sacrificed after 4 weeks.

The skeletons were dried and ashed for aluminum determination. Post-mortem iliac-crest bone specimens obtained from patients in advanced renal failure, who had been treated for a known time period with aluminum hydroxide, were similarly prepared for aluminum determination.

The growth rate of the different test groups is shown on Figure 5. Rats on diet plus aluminum hydroxide showed a significant impairment of growth at 3 and 4 weeks ($P < 0.05$). From measurement of the food intake, the amount of aluminum ingested per day by the rats was found to be 6.0-10.0 mg (equivalent to 60 ml aluminum hydroxide suspension/day for an adult 70-kg man).

It was found that uremic animals showed significantly higher skeletal aluminum levels than the test animals or controls. Patients showed bone-aluminum levels of the same order as the rats, independent of the duration of aluminum hydroxide therapy (see tables in original paper).

The authors remark that their mice did not develop the periorbital bleeding described by Berlyne et al. (014). They attribute this pathology to sick animals and not to the toxic effect of the administered aluminum salts. They also criticize the claims of Berlyne et al. (014) that aluminum is toxic to uremic rats on the basis that the toxicity appeared to depend on either the oral administration of astringent aluminum salts or intra-peritoneal injection of aluminum hydroxide.

They conclude on the basis of their own study that:

(1) Even though aluminum hydroxide impaired the growth rate of normal rats and produced rachitic bone changes, phosphate supplements corrected this effect.

(2) In hyperphosphatemic uremic rats no growth impairment or other pathological abnormalities were observed.

Thus while they have found that in chronic renal failure small quantities of aluminum are deposited in bone, they feel that their work indicates that aluminum hydroxide is nontoxic if hypophosphatemia is avoided.

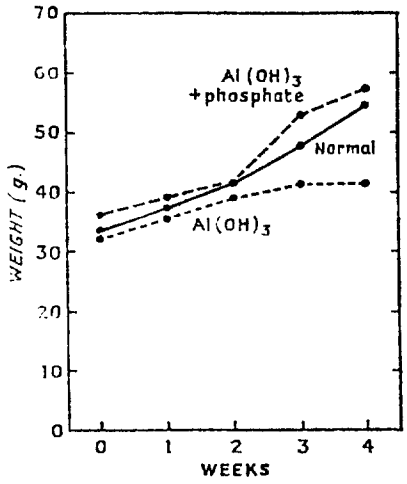

Fig. 5 . Growth of rats treated with aluminum hydroxide or aluminum hydroxide with supplemental phosphate compared with growth rate in normal animals.

7. One month following (May 1972) the paper of Thurston and co-workers (179, excerpted in Section II, B, 6), Berlyne et al. (015) responded in a letter published in The Lancet. They made the following points with respect to the periorbital bleeding:

(1) Their rat colony was healthy, with more than 1000 rats per day in the colony. For 3 years there was not a single case of periorbital bleeding in any rats other than those receiving aluminum salts.

(2) Periorbital bleeding is related to plasma-aluminum levels and occurs at much higher levels than those obtained in rats on oral aluminum hydroxide (see table in original paper, 14, which shows relationship between mode of administration, aluminum salt, and periorbital bleeding).

(3) Because rats have messy eating habits, Thurston et al. (179) could not possibly accurately determine the actual quantity of aluminum consumed in the meal diet they gave their rats.

In conclusion, Berlyne et al. restated that periorbital bleeding is the most reliable sign of aluminum toxicity in rats whose plasma levels approach those of patients with advanced renal failure receiving 6-8 g $Al(OH)_3$/day. Therefore, contrary to Thurston and co-workers (179), Berlyne advises that the use of aluminum salts in acute renal failure should be approached with caution and scientific detachment.

8. Two 90-day subacute oral toxicity studies were carried out by Industrial Bio-Test Laboratories, Inc. (166 and 167) for two sodium aluminum phosphate compounds produced by the Stauffer Chemical Co.--Levair, an acidic salt and Kasal, a basic salt. For the experiments, each salt

was mixed with standard rat ration. The animals were divided into four groups for each test material (Charles River strain albino rats, 15 male and 15 female in each group) as follows: (a) control diet rations only, (b) diet ration plus 0.3% aluminum test material, (c) diet ration plus 1.0% test material, (d) diet ration plus 3% test material. The diets were fed ad libitum and the animals weighed at regular intervals and sacrificed after 90 days. The following were studied: mortality and reactions, hematology, clinical blood chemistry, urine, pathology (gross and microscopic), organ weights, and ratios of organ to body and organ to brain weights. (See the original reports for details and tables.)

Both reports state that there were no significant differences found in any of the studies performed between the test animals and controls, with one exception. At all three dose levels microconcretions were found in the renal tubules of the female rats. These concretions, located in the tubules at the corticomedullary junction, were blue in color and probably calcified. The report concludes that since these concretions were absent in the controls and since their incidence and severity were dose related, they were related to the test material.

C. Chickens

1. Deobold and Elvehjem (039) studied the effect of feeding large amounts of a soluble aluminum salt to chickens. Four groups of day-old white Leghorn chicks (sex and number not given) were fed a special diet ration plus aluminum sulfate (0.44% Al). An amount of sodium acid phosphate was added sufficient to unite with the aluminum in the diet of one of the four groups. (See Table 12 for dosages.) The left tibia was used for bone analysis.

Table 12

Effect of Aluminum Salts on Bone Ash and Blood Phosphorus

Group No.	Salts in ratios			Bone ash	P per 100 ml serum	Ca per 100 ml serum
	$Al_2(SO_4)_3 18H_2O$	Al	Na_2HPO_4			
1	5.37	0.44		31.74	3.5	10.6
2	4.03	0.33		24.61	2.4	12.3
3	2.69	0.22		25.98	2.3	14.0
4	5.37	0.44	5.77	44.72	6.2	10.5

It was observed that:

(1) The chickens fed the diet plus aluminum sulfate all developed severe rickets in 1 or 2 weeks and died within 3 weeks.

(2) The bone ash was reduced to about 25% and the blood phosphorus to 2-4 mg/100 ml serum at about 3 weeks of age or when the chicks had been on the rations for 11 days. This occurred at the level of aluminum salt equivalent to 0.75 of the amount needed to unite with the total phosphorus in the ration (as $AlPO_4$).

(3) When sufficient sodium acid phosphate was added to the diet to unite with the Al, there was no deleterious effects but rather rapid growth and normal bone formation.

(4) After the fifth day of ingesting the aluminum salt, a definite drop in blood phosphate was seen; by the ninth day the values were as low as 1.5-1.7 mg/100 ml serum.

2. Williams and Rodbard (196) studied the effect on young chicks of ingesting reactive aluminum hydroxide gel. Seven experiments were performed with newly hatched commercial incross-bred (Hyline) or New Hampshire chicks. Controls (number not given) were fed a starter mash and experimental animals the same mash mixed with an aluminum hydroxide gel (25 mg/g food). In two of the experiments lower concentrations (3 or 8 mg Al/g food) of the gel were also tested. The following was observed for the chickens on the test diets as compared to controls:

(1) At the highest concentration, weakness manifested by an inability to stand erect was observed within 10 days, more frequently by the third or fourth weeks. Retarded skeletal development without malformation was shown by X-rays. This syndrome could be reversed

only if chicks were returned to a diet free of the Al gel within 1 or 2 days after onset of weakness.

(2) Weight gain was slower than for controls.

(3) At the higher dosage levels there was a high incidence of mortality; all animals died within the first month.

(4) The plasma levels of potassium, carotene, and vitamin A were consistently lower.

(5) Autopsy showed a notable absence of fat deposits, darker-red livers, abnormally small spleens, presence of urate crystals in renal calyces and ureters.

(6) The lower doses did not result in weakness or death.

Further observations by the authors were:

(1) Older animals showed none of the deleterious effects seen in the younger chicks.

(2) A nonreactive aluminum hydroxide gel and an aluminum phosphate gel given in the same high concentrations (25 mg Al/g food) did not produce the syndrome to any great extent.

3. The same group of researchers (Steinborn, Rodbard and Williams, 176) experimented further to determine the mechanism of the weakness produced in young chicks by aluminum hydroxide gel. Three series of experiments were carried out with newly hatched male New Hampshire chicks. In the first experiment 135 chicks were divided into three groups: (a) controls on mash diet, (b) mash diet plus aluminum hydroxide gel (Amphojel, 25 mg Al/g food), (c) same number as in (b) given the same diet but with twice daily injections of a phosphate suspension into the leg muscles.

The observations of the test animals compared to controls were:

(1) Test animals both with and without phosphate injections ate significantly less.

(2) Weight gain for both was also considerably less but phosphate-injected birds gained at a faster rate.

(3) Slightly retarded bone development and poor mineralization showed only on X-rays of test animals on gel diet who did not receive phosphate.

(4) The inorganic serum phosphate of test animals who did not receive injections fell from 7 mg% to 2 mg% in the first week. The serum iron was low in all groups on the gel diet.

(5) Typical weakness developed only in test animals on the gel diet who did not receive phosphate.

(6) The mortality rate for phosphate-injected chicks improved over the other test group.

The second series of experiments with 147 birds was similar to the first and produced similar results. In this series 49 gel-fed chicks were injected with fat vehicle for phosphate without the phosphate. Their survival was even poorer than that of the untreated birds.

In a third series of experiments (120 chicks), injections of phosphate were withheld from animals on a gel diet until weakness had already appeared (7 days). It was found that the phosphate injections improved the survival rate even when started after the onset of weakness symptoms.

The authors concluded that their study, along with others they discuss in the paper, confirms the relation between phosphorus deficiency and certain weakness symptoms in several species, particularly during rapid growth.

4. Pragay (140) found that chicks (number, sex, strain not stated) developed dystrophy (inability to stand or walk) when fed a diet containing a reactive aluminum hydroxide gel (amount unstated, Amphojel). Dystrophic chicks showed lower serum inorganic phosphate and iron as well as cholesterol and vitamin A. (A lower plasma vitamin A was also observed by Williams and Rodbard, 146, Section II, C, 2.)

Other changes noted in chicks who developed dystrophy after an aluminum gel diet were:

(1) Consistently elevated serum alkaline phosphatase and plasma creatinine (30-80%).

(2) Slight elevation of globulins, persistent decrease of lipoproteins.

(3) Decrease in actomyosin muscle content and myosin ATPase activity.

(4) Fragile erythrocytes.

(5) Poor mineralization of bone.

(6) Muscle tissue showed swelling, vacuolization, breaks of the fibers, disappearance of cross-striation, fraying of fiber ends, and fragmentation of nuclei.

D. Rabbits

1. Seibert and Wells (162) studied the toxic effects of various aluminum salts when injected intravenously and when fed. Rabbits (age and sex not given) were used in all the experiments, summarized below:

(1) Five rabbits were given daily ear vein injections of 0.04 g sodium aluminum sulfate [$Al_2(SO_4)_3 \cdot 24H_2O$]. After 2 or 3 weeks they became anemic. The precentage of hemoglobin decreased and in all but one rabbit the number of red corpuscles also decreased.

(2) Four rabbits were given daily ear vein injections of 0.01 g sodium chloride ($AlCl_3 \cdot 6H_2O$). The same decrease in percentage of hemoglobin as with the sodium aluminum sulfate was observed.

The chief pathological changes noted which were attributable to the injections were severe pigmentation, thrombosis, necrosis, and fibrosis in the spleen, and vacuolation and granular degeneration of renal epithelium in the kidney.

(1) In feeding experiments, eight rabbits were fed daily a gelatin capsule containing 0.1 g sodium aluminum sulfate mixed with lactose. Four rabbits were fed up to 60 days and the other four from 150 to 350 days. The percentage of hemoglobin decreased in all but two animals.

(2) Larger doses of aluminum chloride (0.5-10 g) fed to a rabbit over a 14-day period resulted in a decrease of 18-20% in the hemoglobin content of the peripheral blood and a fall of over 3,000,000 in the number of erythrocytes. Another rabbit fed 1.5 g of aluminum chloride died in 2 hours. Pathological examination showed fatty degeneration of the liver, kidneys, and heart in both these animals.

(3) A suspension of aluminum hydroxide fed by stomach tube over 2-5 hours to another rabbit resulted in a drop of 20% in hemoglobin and 2,600,000 in erythrocytes. No pathological changes as in other experiments were observed on autopsy.

The authors conclude that:

(1) Aluminum compounds are both toxic and lethal when put in direct contact with blood and tissues.

(2) Ingesting small daily doses of aluminum compounds in any form (even one presumably insoluble and inert like aluminum hydroxide) produced changes in the blood and tissues which indicate that aluminum is absorbed through the intestinal tract.

(3) Aluminum is particularly toxic to red corpuscles. The authors suggest the possibility that analogous to lead poisoning, years of slow absorption of minute amounts of aluminum are required to effect the same results that are accomplished in a short time by injecting an aluminum compound directly into the blood stream.

E. Dogs

1. Two 90-day subacute oral toxicity studies were carried out by Industrial Bio-Test Laboratories, Inc. (168 and 169) for two sodium aluminum phosphate compounds produced by the Stauffer Chemical Co.--Levair, an acidic salt and Kasal, a basic salt. For the experiments, each salt was mixed into a stock diet. The dogs were divided into four groups for each test material (4 male and 4 female purebreed beagle dogs in each group) as follows: (a) control, stock ration only, (b) stock ration plus 0.3% test material, (c) stock ration plus 1.0% test material, (d) stock ration plus 3.0% test material. The food was fed ad libitum. The dogs were regularly observed and examined and sacrificed at conclusion of the study. The following were studied: food consumption, body weight, hematology, blood chemistry, urine, pathology (gross and histopathologic), and organ weights (see the original reports for details and tables).

Both reports state that there were no significant differences found in any of the studies between the test animals and controls, with one exception. The study on Kasal (sodium aluminum phosphate basic) reports

finding unusually large and numerous renal concretions in three of the test dogs (fed 3% test material) which were considered to be related to the experiment. It is of interest that the similar study with Levair (sodium aluminum phosphate acidic) did not report this finding in any of the dogs whereas the same type of study with rats (see Section II, B, 8) reported finding renal concretions with both these test materials.

F. Humans

1. Havens (071) reports the case of a patient with a bleeding duodenal ulcer who upon death showed an intestinal obstruction caused by colloidal aluminum hydroxide. Havens warns that this danger exists particularly with older or very ill patients whose energy is depleted or whose intestinal tract may lack normal tonus.

2. Levy (105A) reported a case of a man who was gradually being poisoned by the addition of alum (potassium aluminum sulfate) to his food by his wife. The man recovered when he left home and ate an alum-free diet.

3. Kirsner (090) studied the influence of aluminum hydroxide on the acid-base balance and on renal function by administering varying quantities of aluminum hydroxide (Amphojel) to 23 patients with peptic ulcers. These patients had taken 31-393 mg of Amphojel daily for up to 8 months for therapy. The blood urea nitrogen and urea clearance test for renal function were determined in 14 of these patients. The carbonic acid content and pH of the blood plasma remained within normal limits. No disturbance in acid-base balance or renal function was observed.

The authors conclude that aluminum hydroxide, even in large amounts, is safe for individuals with a marked reduction in renal efficiency.

4. On the other hand, Bloom and Flinchum (019) suggest that aluminum hydroxide ingestion may not be as innocuous as has been reported. They report the case of a patient with severe osteomalacia with pseudofractures, who ingested large amounts of aluminum hydroxide. They note that serum phosphate fluctuates with aluminum hydroxide ingestion, as demonstrated in their patient. Furthermore, they cite a study on phosphate retention in children with chronic renal deficiency in which it was found that a lowering of the level of phosphate in the extracellular fluid and serum was a function of the time and amount of aluminum hydroxide ingested.

They conclude by urging that a more careful search be made for patients with metabolic bone disease resulting from aluminum hydroxide ingestion and warning that unless phosphate changes in patients ingesting aluminum hydroxide are monitored, it could be a more potentially dangerous medicine than has been realized.

5. In a later study reported by Lotz et al. (109), further evidence is given that antacids can impair phosphorus absorption in man. A combination of magnesium aluminum hydroxide (60 ml) and aluminum hydroxide (30 ml) was given four times a day by mouth to six patients (3 normal, 2 with hypoparathyroidism and one with pseudohypoparathyroidism) for 130 days. All subjects so treated manifested a debility characterized by weakness, anorexia, bone pain, and malaise. The syndrome of phosphorus depletion was also shown by hypophosphatemia, hypophaturia, increased gastrointestinal absorption of calcium, hypercalciuria, increased resorption of skeletal calcium and phosphorus. Theraphy simply involves providing adequate dietary phosphorus.

The authors warn that use of antacids with patients receiving corticosteroids may cause phosphorus depletion. They conclude that prolonged excessive ingestion of certain antacids produces a syndrome of clinical importance in man attributable to phosphorus depletion.

III. Long-Term Studies

Rats

Lyman and Scott (111) studied the effects on the growth and kidney structure of albino rats fed two types of baking powders with their diet (sodium aluminum sulfate and tartrate baking powder). The details on this experiment are written up in Biological Section II, B, 1. Some of the rats (number not stated) were kept on the diet containing about 2% S.A.S. baking powder for a maxmimum of 21 months. The kidneys of these rats could not be distinguished in gross or microscopic pathology from those of the controls.

IV. Special Studies

A. Humans

1. Berlyne *et al.* (013) studied the effect of ingestion of aluminum-cycle resins and aluminum hydroxide on patients with advanced renal failure. Plasma aluminum levels were found to be greatly raised in one out of every three persons taking aluminum-cycle resins or aluminum hydroxide as well as in patients being dialyzed. The elevation of serum aluminum levels was also noted in some dialyzed persons who were ingesting aluminum hydroxide regularly.

The authors point out that the toxic effects of this hyperaluminemia which apparently result from the ingestion of aluminum salts and aluminum

cycle resins or from dialysis fluid, are unknown in man. Much of the published work on animals is related to the effects of concomitant phosphate depletion resulting from the administration of aluminum hydroxide (see Biological Section II, B 5, C 2, 4, and F 5). The authors therefore caution against the use of aluminum resins and salts in persons with renal failure until serum aluminum levels can be measured and monitored accurately and more is known about the potential toxic effects of hyperaluminumemia.

2. Following the Berlyne et al. (013) article on hyperaluminemia, an exchange of letters appeared in The Lancet. Wrong and Swales (200) question the toxicity of aluminum preparations when used to treat patients with renal failure with hyperphosphatemia. Berlyne (012) responded by asserting that Wrong and Swales had missed the point of his article (013), namely, that aluminum resins cause hyperaluminemia in 30% of renal failure patients. Whether or not hyperaluminemia has toxic effects, however, requires further research. For this reason Berlyne cautions that until serum aluminum levels can be measured easily, aluminum resin should be regarded with some suspicion.

3. Erdohazi and Newman (043) describe two cases in humans of granulomas produced by injection of a vaccine containing aluminum hydroxide adjuvant. The authors discuss other reports of cases of granulomas resulting from aluminum oxide vaccines. They consider their two cases of interest because of the conclusive evidence in one of the presence of aluminum salts and the strongly histological evidence in the other suggesting a similar presence.

BIOCHEMICAL SECTION

I. Breakdown

The breakdown of the various aluminum compounds in the body is discussed in the following sections. No breakdown in storage was noted.

II. Absorption and Distribution

Introduction

Before sensitive analytical methods were developed for determination of aluminum, it was thought by a number of researchers (in the 1920's and 1930's) that ingested aluminum is not absorbed but is quantitatively excreted in the urine and feces (117, 112, 181, and 201). Certain authors, such as Underhill and co-workers (183, 184, 186, and 188), however, had reported finding ingested aluminum deposited in small amounts in the liver, brain, kidneys, spleen, and thyroid, with the bile being the most important pathway for excretion. Later studies (1967) showed that a large increase in aluminum intake results in increased absorption of aluminum which is deposited in certain tissues (skeleton and liver) and partly eliminated by the urine (134, 133). Thus it was shown that the amount of aluminum retained in the body depends on the quantity in the food.

A. Rats

1. Ondreicka et al. (134) demonstrated increased retention of aluminum when high doses of an aluminum salt are ingested. One group of eight rats (Wistar strain, 150-250 g) was fed a basic diet containing 160-180 ppm Al (as aluminum sulfate) and another group the same diet containing 2,835 ppm Al (as aluminum sulfate) for 24 days. The con-

siderably increased doses of aluminum sulfate (200 mg Al/kg) resulted in increased tissue concentrations, particularly in the liver, testes, and bone, while in the other organs the aluminum level was not significantly changed (see Table 13). The experimental data show that, because of increased fecal elimination, there is no significant increase in aluminum retention when there is only a slight increase in the ingestion of aluminum compounds. Higher amounts of aluminum intake, however, result in increased absorption in some tissues and elimination via the urine. The authors conclude that the concentration of aluminum in the body is a function of the amount of aluminum in the food.

2. Ondreicka et al. (133) have also shown that the retention of aluminum is dependent on the composition of the food, specifically that a high fluoride content decreases aluminum retention. The authors describe the results of balance experiments with rats in which they observed that:

(1) After 1 mg fluorine (as calcium fluoride) per animal was given, the elimination of aluminum in the urine and feces was significantly increased and retention was decreased.

(2) Aluminum retention in bone was decreased by increasing doses of fluoride and prolonging administration time.

In one series of experiments rats were simultaneously given Al (50 mg/kg) and fluorine (1 mg F as calcium fluoride) perorally per animal for 24 days. As can be seen on Table 14, following the administration of Al alone, the level in all the organs and tissues except the intestines is higher. Administering fluoride resulted in decreased aluminum levels in most organs. When fluoride and aluminum were given simultaneously, there was no substantial difference in aluminum level compared with that in the control

Table 13

Influence of Dose Rates on Aluminum Levels in Rat Tissues[a]

Tissue	Concentration of Al in diet 170 ppm	2835 ppm	Statistical significance
Liver	1.55 ± 0.26	2.99 ± 0.28	$P < 0.001$
Brain	0.71 ± 0.08	1.08 ± 0.15	$P < 0.05$
Kidneys	0.86 ± 0.08	0.90 ± 0.10	$P > 0.05$
Adrenals	41.5 ± 5.2	51.4 ± 5.5	$P > 0.05$
Spleen	1.46 ± 0.23	1.48 ± 0.10	$P > 0.05$
Heart	1.08 ± 0.12	1.27 ± 0.12	$P > 0.05$
Lungs	0.407 ± 0.054	0.428 ± 0.080	$P > 0.05$
Testes	0.170 ± 0.057	0.470 ± 0.060	$P < 0.001$
Colon	0.69 ± 0.10	0.63 ± 0.04	$P > 0.05$
Muscle	0.039 ± 0.008	0.044 ± 0.002	$P > 0.05$
Blood	0.65 ± 0.11	1.08 ± 0.09	$P < 0.01$
Femur	70.2 ± 1.0	91.2 ± 0.7	$P < 0.001$

[a]Aluminum concentrations are given in mg/100 g fresh tissue. The diet was maintained for 24 days before the rats were killed.

Table 14

Aluminum Levels in Some Rat Tissues and in Blood After Administration of Increased Doses of Al^{3+} and F^-

Tissue	Larsen diet	Larsen diet +50 mg Al	Larsen diet +1 mg F	Larsen diet +50 mg Al +1 mg F
Liver	1.55 ± 0.25	2.99 ± 0.27	1.46 ± 0.11	1.90 ± 0.21
Brain	0.71 ± 0.08	1.07 ± 0.15	0.66 ± 0.09	0.77 ± 0.11
Kidneys	0.86 ± 0.08	0.90 ± 0.10	0.73 ± 0.10	0.85 ± 0.09
Spleen	1.46 ± 0.22	1.48 ± 0.10	1.25 ± 0.12	1.43 ± 0.21
Heart	1.07 ± 0.12	1.27 ± 0.12	1.00 ± 0.14	1.05 ± 0.12
Lungs	0.40 ± 0.05	0.42 ± 0.08	0.42 ± 0.06	0.41 ± 0.06
Testes	0.17 ± 0.05	0.47 ± 0.06	0.17 ± 0.02	0.21 ± 0.04
Colon	0.68 ± 0.10	0.62 ± 0.04	0.59 ± 0.06	0.60 ± 0.09
Muscle	0.03 ± 0.008	0.04 ± 0.002	0.04 ± 0.007	0.04 ± 0.005
Blood	0.65 ± 0.11	1.10 ± 0.08	0.79 ± 0.06	0.82 ± 0.10
Femur	70.18 ± 0.98	91.16 ± 0.65	44.08 ± 0.54	75.42 ± 0.80

group. The authors suggest their results indicate that in the organism aluminum and fluoride form a complex $(AlF_6)^{-3}$ which is more soluble than calcium fluoride, and thus removes aluminum from the tissues. The authors note that as yet the question of the organisms physiological requirement for aluminum is unresolved, particularly in regard to growth and fertility.

B. Dogs

Underhill and Peterman (183) studied the absorption of orally ingested aluminum (as straight alum, sodium aluminum sulfate, and alum phosphate) in dogs. The dogs used in these experiments were of unknown origin but in good health. The experimental dogs were fed meat-containing biscuits made with either sodium aluminum sulfate or alum phosphate baking powder and the controls with a diet containing biscuits made with yeast. Various feeding experiments were carried out with both fasting and nonfasting dogs, with the following results (for details see the tables in the original paper):

(1) The blood of dogs fasted for 7 days was found to contain appreciable quantities of aluminum. The liver, kidney, brain, and spleen were also found to contain relatively large amounts of aluminum. From the large amount in the bile, which was more than eight times that in the blood, the authors deduce that in the fasting animal the liver is the organ most responsible for excreting aluminum, which is probably reabsorbed in large part.

(2) The blood, kidney, brain, and spleen of dogs on a normal diet (no added aluminum) were higher in aluminum than in the fasting dogs. The liver and bile were slightly lower. Thus it would seem that a diet without added aluminum can be a source of

aluminum found in the blood. Flour, which was a constituent of this diet, is high in aluminum.

(3) In subsequent experiments, groups of dogs were given:

(a) A single feeding containing aluminum.

(b) A 1-week aluminum-containing diet.

(c) A 4-week aluminum-containing diet.

(d) A 12-week aluminum-containing diet.

In general, the average aluminum content of the blood decreased when large amounts of aluminum were fed over a protracted period. The absorbed aluminum is highest in the spleen, followed by brain, liver, and kidney. The authors register surprise at the high amount per unit of tissue in the thyroid. The bile is the chief path of excretion. The absorption of aluminum continues but decreases when aluminum-rich diets are fed over a prolonged period. Storage and excretion decrease as absorption decreases.

C. Humans

Underhill and Peterman (184) studied the absorption of aluminum in man. They used 27 young volunteers in two experiments. In the first experiment (15 subjects), 10 subjects were fed a meal with biscuits made with aluminum baking powder and 5 the same meal with yeast bread. In the second experiment the subjects (12 divided into 2 groups) ate a supper, breakfast, and lunch with aluminum biscuit or yeast bread. Blood and urine samples were taken from the subjects before the feeding experiments started. The observations were:

(1) Normal human blood may or may not contain aluminum at any given time. It varies between narrow limits.

(2) After the ingestion of aluminum-rich food, an increase in aluminum is not always detected in the blood. There is some evidence that, as with dogs (183), there is a delay in the absorption of aluminum after eating aluminum-rich foods. The authors speculate that this may indicate that aluminum is more easily absorbed from the lower part of the intestinal tract than from the upper part.

(3) Normal urine may or may not contain aluminum. The aluminum content of urine tends to increase after the ingestion of aluminum-rich foods. The fact that the urine shows definite amounts of aluminum indicates that it must be present at times in the blood, even if it is not always detected. (See tables in original paper for details of these results.)

III. Metabolism and Excretion

A. Mice

Ondreicka et al. (134) confirmed the interaction of aluminum with phosphorus in the gastrointestinal tract. Ten mice (Dobra-Voda strain, 200 g) were fed a standard diet plus 160-180 ppm Al (as aluminum chloride) and ten mice were fed the same diet plus 355 ppm Al (as aluminum chloride) for 40 days. Intake and fecal excretion were significantly higher at the higher dose. Urinary excretion and aluminum retention were not significantly higher. The higher dose significantly lowered phosphorus retention, which on some days became negative, i.e., there was net excretion.

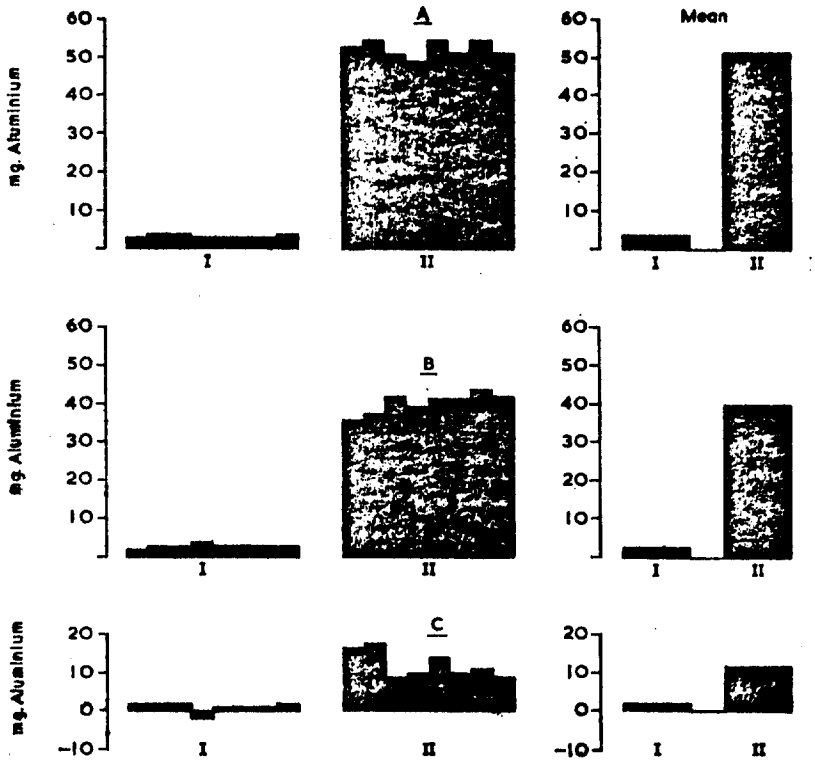

Fig. 6. Influence of high doses of aluminum sulfate on the aluminum balance in rats: A, aluminum intake; B, aluminum excretion; C, aluminum retention: I, control group (170 ppm Al in diet); II, treated group (2835 ppm Al in diet). All values are expressed in mg Al.

Table 15. Influence of Aluminum Trichloride Intoxication on the Tissue Incorporation and Excretion of ^{32}P in Rats[a]

Sample	Control (cpm)	Single oral dose of 188 mg/kg (cpm)	Statistical significance
Blood	982 ± 73	77 ± 11	$P < 0.001$
Liver	6,891 ± 936	342 ± 52	$P < 0.001$
Spleen	3,934 ± 306	140 ± 29	$P < 0.001$
Kidneys	4,644 ± 489	327 ± 69	$P < 0.001$
Brain	816 ± 75	190 ± 61	$P < 0.001$
Muscle	1,986 ± 165	33 ± 10	$P < 0.001$
Femur	23,390 ± 2,040	950 ± 280	$P < 0.001$
Feces/24 hours	1,799,000 ± 378,000	1,974,000 ± 565,000	$P > 0.05$
Urine/24 hours	1,195,000 ± 197,000	20,750 ± 4,190	$P < 0.001$

[a]Average values ± S.E. are given.
For blood the count rates are expressed per 100 µl, for tissues per 100 mg, and for feces and urine, the total excreted in 24 hours.

removed from the area of absorption. Eighteen rats were divided into three groups (6 in each) as follows: (a) controls, (b) animals given daily oral doses of aluminum chloride (36.5 mg Al/kg) for 52 days, (c) animals fed a standard diet for 52 days, then given a single large intragastric dose of aluminum chloride (240 mg Al/kg). The $Na_2H^{32}PO_4$ was injected intraperitoneally (0.16 μC/100 g). It was found that both chronic and acute peroral dosing with aluminum chloride led to a decrease in the incorporation of ^{32}P into the phospholipid fraction as well as into the ribonucleic and deoxyribonucleic acids in various tissues (see Table 16).

The authors conclude that since phosphorus incorporation into phospholipid and nucleic acids depends on the activity of the phosphorylating mechanisms, it seems evident from this experiment that dosing with aluminum salts disturbs the activity of phosphorylating mechanisms.

4. Since adenosine triphosphate is an important component of the phosphorylating mechanisms, Ondreicka et al. (134) studied the effect of chronic and acute dosing with aluminum chloride on the blood serum level of adenosine mono-, di-, and triphosphates. Thirty rats were divided into three groups of ten each, with all receiving the standard diet for 55 days: (a) controls; (b) animals given additional aluminum chloride (36.5 mg Al/kg/day) for the 55 days; (c) animals given one oral dose of aluminum chloride (223 mg Al/kg) on the 56th day. The results (see Table 17) show that the concentration of adenosine triphosphate in the rats' serum was decreased and that of adenosine di- and monophosphate increased. This significant decrease in the ATP/ADP ratio indicates that the equilibrium in the system

$$ATP \rightleftarrows ADP + \text{inorg P} \rightleftarrows AMP + \text{inorg P}$$

Table 16

Influence of Chronic and Acute Aluminum Trichloride Intoxication on the Incorporation of ^{32}P into Tissue Fractions in the Liver, Spleen, and Kidneys of Rats[a]

Fraction	Specific activity (counts/min/100 μg P)			Significance against control	
	Control	Chronic intoxication	Acute intoxication	Chronic intoxication	Acute intoxication
Liver					
Acid-soluble P	1944 ± 96	1806 ± 81	2244 ± 132	$P > 0.05$	$P > 0.05$
Lipid P	2139 ± 108	1720 ± 132	1625 ± 61	$P < 0.05$	$P < 0.01$
RNA	1234 ± 65	928 ± 40	870 ± 207	$P < 0.01$	$P > 0.05$
DNA	176 ± 16	172 ± 23	95 ± 15	$P > 0.05$	$P < 0.01$
Spleen					
Acid-soluble P	2395 ± 177	1997 ± 112	1736 ± 356	$P > 0.05$	$P > 0.05$
Lipid P	1294 ± 176	986 ± 55	744 ± 271	$P > 0.05$	$P > 0.05$
RNA	1671 ± 504	1242 ± 119	358 ± 131	$P > 0.05$	$P < 0.01$
DNA	2285 ± 1324	1750 ± 227	252 ± 62	$P > 0.05$	$P < 0.01$
Kidneys					
Acid-soluble P	1994 ± 57	1801 ± 83	2238 ± 95	$P > 0.05$	$P > 0.05$
Lipid P	1743 ± 194	1190 ± 77	1013 ± 61	$P < 0.05$	$P < 0.05$
RNA	834 ± 65	696 ± 57	602 ± 69	$P > 0.05$	$P < 0.05$
DNA	128 ± 28	334 ± 152	44 ± 8	$P > 0.05$	$P < 0.05$

[a]Average values ± S.E. are given.

Table 17

Influence of Chronic and Acute Intoxication by Aluminum Chloride on the Level of Adenosine Mono-, Di-, and Triphosphates in Rat Blood[a]

Acid	Control	Chronic intoxication	Acute intoxication	Significance against control: Chronic intoxication	Significance against control: Acute intoxication
AMP (mg/100 ml)	2.95 ± 0.19	3.18 ± 0.60	3.48 ± 0.27	$P > 0.05$	$P > 0.05$
ADP (mg/100 ml)	3.69 ± 0.19	4.50 ± 0.42	4.39 ± 0.11	$P > 0.05$	$P < 0.01$
ATP (mg/100 ml)	8.33 ± 0.41	7.56 ± 0.95	5.52 ± 0.44	$P > 0.05$	$P < 0.001$

[a]Average values ± S.E. are given.

is shifted to the right. The mechanism of this shift is not known. However, the experiments of Jones (082) and Pragay (140) (see Biological Section II, B 2, and C 2) indicate that it may be connected with a decrease in the level of inorganic phosphate in the blood of animals intoxicated by aluminum compounds. The authors point out that decreased production of ATP could endanger the course of a whole series of phosphorylation reactions, such as the synthesis of phospholipids and nucleic acids.

5. Because the metabolism of carbohydrates is closely interrelated with phosphorylation mechanisms, Ondreicka et al. (134) report on several studies carried out by themselves and other researchers. When aluminum chloride (122 mg Al/kg BW) was administered intragastrically to rats, the concentration of liver glycogen decreased to as low as one-fourth the control values; aldolase activity in the blood serum increased by more than twice the normal values and some animals showed definite hyperglycemia. The authors comment that the total character of the metabolic changes in aluminum-intoxicated rats appeared to resemble disturbances in carbohydrate metabolism which occur as a result of the action of hepatotoxic substances. When lower doses of aluminum chloride (69 mg Al/kg BW) were given to rats over a longer period, carbohydrate metabolism was affected to a lesser degree. A trend toward decreased hepatic and muscular glycogen was seen.

When the dose of aluminum salt was tripled (200 mg Al/kg BW), the observations were:

(1) A decrease in liver glycogen to nearly one-tenth normal level.

(2) A significant decrease in skeletal muscle glycogen.

(3) An increase in lactic acid in both liver and muscle.

(4) A significant increase in pyruvic acid concentration in blood serum and liver.

(5) A decrease in coenzyme A activity in the liver to less than one-third normal values.

(6) Animals dosed with high levels of aluminum salt decreased their food intake with a consequent weight reduction.

The authors mention a recent finding that aluminum salts inhibit absorption of glucose from the intestinal tract. They conclude that these significant changes in carbohydrate metabolism found to accompany aluminum intoxication are probably mediated by changes in phosphorus absorption.

6. Vozar (190) investigated the effect of aluminum compounds on glycide metabolism. Two different aluminum compounds were studied--a solution of aluminum chloride and a suspension of aluminum oxide. Both were administered daily by probe in 1.5-ml physiological solution to groups of 12 rats (Wistar strain, 125-175 g) for periods of 5 and 10 days. The daily dose of both compounds was equivalent to 15 mg Al/100 g BW. The controls received an equal amount of physiological solution. The following was observed:

(1) For animals receiving aluminum chloride

(a) A rise in glycogen in the liver and skeletal musculature after 5 days and in the liver only after 10 days.

(b) No changes in glycemia values were found.

(2) For the animals receiving aluminum oxide, the opposite was found. There was a decline in glycogen and glycemia values in the liver and skeletal musculature after both 5 and 10-day feeding periods. (For details see Tables 18 and 19.)

The author concludes that orally ingested aluminum compounds impair the sequence of biochemical processes in the metabolism of glycides as seen

Table 18

Glycogen and Glycemia Levels in Control Rats and Rats Regularly Fed 15 mg Al/100 g BW of $AlCl_3 \cdot H_2O$ for 5 and 10 Days

Period of feeding (days)	Group of rats	No. of rats	Liver (g%)	S.E.	Skeletal musculature (g%)	S.E.	mg%	S.E.
1	2	3	4	5	6	7	8	9
5	Control	12	1.306	0.137	0.418	0.068	110.07	5.43
	Experimental	12	3.683	0.211	0.440	0.049	103.32	3.86
10	Control	12	0.958	0.108	0.481	0.061	96.18	2.69
	Experimental	12	2,224	0.405	0.583	0.032	98.26	3.72

Table 19

Glycogen and Glycemia Levels in Control Rats and Rats Regularly Fed 15 mg Al/100 g BW of $AlCl_3 \cdot H_2O$ for 5 and 10 Days

Period of feeding (days)	Group of rats	No. of rats	Liver (g%)	S.E.	Skeletal musculature (g%)	S.E.	mg%	S.E.
1	2	3	4	5	6	7	8	9
5	Control	12	1.317	0.181	0.320	0.037	103.08	4.40
	Experimental	12	0.237	0.023	0.124	0.017	91.43	1.61
10	Control	12	0.975	0.086	0.251	0.019	101.07	3.31
	Experimental	12	0.304	0.028	0.120	0.012	102.32	3.77

in the observed changes in the organisms' glycide reserves. He points out that the difference in the action of aluminum chloride and aluminum oxide is owing to the fact that the former compound was diluted in water and the latter was undiluted. The disturbance in glycide metabolism is attributed to a malfunction in phosphorus metabolism caused by the ingestion of aluminum salts.

7. Kortus (100) studied the effect on carbohydrate metabolism of feeding high doses of aluminum chloride. Two groups of 8 white male rats (Wistar, 175 $\pm$ 10 g) received a basic diet. The experimental animals were fed aluminum chloride (200 mg Al/kg) daily for 18 days. It was estimated that about 10% of the administered salt was absorbed. The results are summarized in Table 20. The author concludes that the results (decrease of liver glycogen and increase of lactic and pyruvic acids) indicate a disturbance of glycide metabolism accompanying the ingestion of the aluminum salt, resulting in decreased glucose absorption from the gut. He speculates that a disturbance in phosphorus metabolism provoked by large doses of aluminum salt is the underlying cause. (See Section III, B, 3.)

C. Humans

1. Kirsner (093) studied the action of ingested aluminum hydroxide and aluminum phosphate on mineral excretion in humans. He found in a study with three patients that ingesting either aluminum phosphate gel (650 mg Al/24 hours) or aluminum hydroxide caused an increase of phosphorus content in the feces but not in the urine, which remained unchanged. This is further evidence of the relation of aluminum to phosphorus metabolism.

2. Child et al. (029) describe the case of a patient with a chronic duodenal ulcer who developed concretions after the ingestion of aluminum

Table 20

Influence of Aluminum Intoxication on Experimental Rats

Values estimated No. of rats	Control group 8	Experimental group 8	Significance
Blood glucose, mg%	109.60 ± 4.25	103.30 ± 2.23	$P > 0.05$
Glycogen			
Liver, g%	2.530 ± 0.085	0.309 ± 0.030	$P < 0.001$
Muscle, g%	0.442 ± 0.028	0.271 ± 0.023	$P < 0.001$
Lactic acid			
Blood, mg%	13.72 ± 0.76	13.55 ± 0.66	$P > 0.05$
Liver, mg%	11.89 ± 0.75	15.36 ± 0.72	$P < 0.01$
Muscle, mg%	32.36 ± 3.50	51.61 ± 2.80	$P < 0.001$
Pyruvic acid			
Blood, mg%	0.265 ± 0.012	0.366 ± 0.019	$P < 0.001$
Liver, mg%	0.296 ± 0.002	0.400 ± 0.021	$P < 0.001$
Coenzyme A, liver	17.27 ± 1.03	5.05 ± 0.27	$P < 0.001$

hydroxide. These small concretions, which caused pain on passage through the intestine, were found on analysis to be composed of aluminum salts of fatty acids, with a small amount of neutral fat and traces of cholesterol and protein.

3. Bailey et al. (006) studied the effect of aluminum hydroxide on the calcium, phosphorus, and aluminum balances and on the serum parathyroid hormone of patients with advanced renal failure. Eight patients with chronic renal failure were given 75-150 ml aluminum hydroxide (1.5-3.4 g Al) daily. All patients were found to absorb and retain aluminum. Plasma phosphorus fell in all patients. The authors explain the fall in plasma phosphorus after the administration of aluminum hydroxide by suggesting that aluminum combines with phosphate after absorption and is deposited in bone.

IV. Effects on Enzymes and Other Biochemical Parameters

A. Rats

1. Berlyne et al. (014) showed that aluminum compounds depress rat liver respiration. Table 21 gives the details of the experiment. The rats used were nonnephrectomized littermate white adult male rats (Weizmann Institute strain, 200 g). The test with aluminum hydroxide used livers from rats receiving the salt either orally or intraperitoneally (3 ml/day, equivalent to 90 mg/kg BW/day). Controls drank tap water. The test was repeated by adding aluminum sulfate (various concentrations up to 40 mg/liter) to normal rat liver homogenate.

Table 21

In Vitro Oxygen Uptake in Liver Homogenates of Rats

Group[a]	Preparation of liver homogenate	No. of rats	Mean (and range) oxygen uptake (µl/min mg protein)
1	i.p. $Al(OH)_3$	5	1.98[b] (1.56-2.36)
	Control	5	2.67 (2.01-3.71)
2	*In vitro* addition of $Al_2(SO_4)_3$	5	1.72[c] (1.22-2.04)
	Control	5	1.79 (1.32-2.07)

[a]Group 1. Treated with $Al(OH)_3$ intraperitoneally, and control group.
Group 2. All livers from normal (untreated) rats. 40 µg per ml aluminum as $Al_2(SO_4)_3$ added to homogenate at start of incubation, nothing added to controls.

[b]Significantly lower than control ($T = 2.78$, $P < 0.05$).

[c]Not significantly lower than control ($T = 2.42$, $0.1 > P > 0.05$).

It was observed that oxygen consumption in aluminum-treated rats fell by 25% (see Table 21). The depression of liver respiration by the action of aluminum salts *in vitro* is indicative of a direct toxic action on the liver cell. The authors are doing further work to elucidate the mechanism. The authors further note that there was a reduction in liver-protein concentration found in nonuremic rats ingesting aluminum hydroxide in drinking water, which may be due to direct interference with protein synthesis.

2. Street (177) studied quantitatively the influence of aluminum on phosphorus absorption. Sodium phosphate and aluminum compounds in various amounts and ratios were added to the diet (adequate except for phosphorus) of young rats (40-50 g, in groups of ten). The aluminum hydroxide used was a powdered commercial tablet mixture (Creamalin tablets).

In experiment I (see Table 22) different groups of animals were fed the diet alone, the diet plus sodium acid phosphate, or the diet plus phosphate and aluminum hydroxide. Since the effect of the aluminum feeding on phosphorus absorption was much smaller than anticipated from the work of previous investigators (6, 504, and 508), two more experiments were carried out to try to explain the discrepancy (summarized in Table 22).

In the second experiment the effect on phosphorus utilization of aluminum sulfate and aluminum hydroxide was compared. The third experiment was carried out to make a more quantitative determination of the effect of aluminum hydroxide on phosphorus utilization.

Table 22

Summary of Experiments I, II and III

Group no.	Material added to basal diet	Phosphorus content of ration (%)	Atomic ratio Al:P	Average gain in weight in 6 weeks (g)	Blood inorganic phosphorus av. value mg/100 ml
	Experiment I				
1	None	0.04		19.8	3.7
2	$NaH_2PO_4 \cdot H_2O$	0.24		105.6	8.2
3	$NaH_2PO_4 \cdot H_2O$	0.60		100.6	9.0
4	$NaH_2PO_4 \cdot H_2O$ + 0.6% $Al(OH)_3$	0.24	1:1	70.6	6.0
5	$NaH_2PO_4 \cdot H_2O$ + 3.0% $Al(OH)_3$	1.20	1:1	72.3	
	Experiment II				
1	None	0.04		11.1	3.54
2	$NaH_2PO_4 \cdot H_2O$	0.20		128.9	7.15
3	$NaH_2PO_4 \cdot H_2O$	0.60		145.2	8.37
4	$NaH_2PO_4 \cdot H_2O$ + 1% $Al(OH)_3$	0.20	2:1	43.3	3.19
5	$NaH_2PO_4 \cdot H_2O$ + 1% $Al(OH)_3$	0.60	2:3	137.7	7.87
6	$NaH_2PO_4 \cdot H_2O$ + 2.58% $Al_2(SO_4)_3 \cdot 18\ H_2O$	0.24	1:1	11.9	3.66
7	$NaH_2PO_4 \cdot H_2O$ + 2.58% $Al_2(SO_4)_3 \cdot 18\ H_2O$	0.60	2:5	142.4	9.00

Table 22 (Continued)

Group no.	Material added to basal diet	Phosphorus content of ration (%)	Atomic ratio Al:P	Average gain in weight in 6 weeks (g)	Blood inorganic phosphorus av. value mg/100 ml
		Experiment III			
1	None	0.04		4.7	2.40
2	$NaH_2PO_4 \cdot H_2O$	0.10		38.4	2.98
3	$NaH_2PO_4 \cdot H_2O$	0.15		95.9	4.46
4	$NaH_2PO_4 \cdot H_2O$	0.20		140.8	4.88
5	$NaH_2PO_4 \cdot H_2O$ + 1% $Al(OH)_3$	0.20	2:1	46.8	2.98
6	$NaH_2PO_4 \cdot H_2O$ + 1% $Al(OH)_3$	0.60	2:3	161.7	7.08
7	$NaH_2PO_4 \cdot H_2O$ + 0.5% $Al(OH)_3$	0.20	1:1	84.2	3.65
8	$NaH_2PO_4 \cdot H_2O$ + 2.58% $Al_2(SO_4)_3 \cdot 18\ H_2O$	0.24	1:1	17.7	2.27

The results of these experiments show that:

(1) When aluminum sulfate was fed in amounts chemically equivalent to the phosphorus in the ration, essentially all of the phosphorus became unavailable.

(2) When aluminum hydroxide was fed at levels of 0.5 and 1.0% of the diet, one-third to one-fourth of the aluminum was converted to a form that reacts with phosphorus.

The author concludes that:

(1) When a soluble form of aluminum (such as aluminum phosphate) is fed in amounts equal to the dietary phosphorus content, nearly complete precipitation of phosphorus occurs in the intestinal tract.

(2) The reaction of the insoluble (in neutral solution) aluminum hydroxide with the phosphate in the intestinal tract is suggested as a result of its partial conversion to aluminum chloride by solution in the acidic contents of the gastric tract.

B. Chicks

1. Bishop et al. (018) studied the ATP level in the erythrocytes of chicks fed aluminum hydroxide. Two similar series of experiments were performed, the second differing from the first only in being carried out on larger groups. Three groups of newly hatched De Kalb chicks (number not given) were treated as follows:

(1) Control group regular mash diet.

(2) Same mash with 25 mg aluminum hydroxide per gram of food added.

(3) Same as (2); chicks also received twice daily intramuscular injections of a fat emulsion of Na_2HPO_4 (approximately 10% phosphorus).

Both groups of chickens developed the characteristic fatal syndrome of leg and wing weakness (see 196 and 176, Biological Section II, C, 2, and 3). In the first series, muscle weakness and death occurred in about 2.5 weeks and in the second series in about 1.5 weeks.

Table 23 shows the ATP levels in the various experimental groups. It was found that the ATP levels of the blood in both experimental series in which aluminum hydroxide gel was administered were markedly reduced. Intramuscular injections of phosphate partially counteracted these effects.

The authors conclude that their results suggest the possibility that muscle weakness in this syndrome may be accompanied by lowered ATP levels in muscle. A general failure of phosphorylation, due to a decreased availability of inorganic phosphate probably takes place since the erythrocytes cannot maintain their ATP levels under the experimental conditions. Partial recovery takes place when more inorganic phosphate is supplied.

C. Rabbits

Schwab and Javillier (160) investigated whether aluminum (in the form of an aluminum salt) would affect the hypoglycemic effect of insulin and the hyperglycemic effect of adrenaline in the same way that zinc (as a zinc salt) does. Two experiments with rabbits (2-3 kg, number not given) previously fasted for 12 hours were carried out. In the first experiment, insulin-gelatin (one unit of insulin plus 2 cc of gelatin in the ratio 1:100) was injected subcutaneously with 3.5 mg aluminum (as aluminum chloride), a strong dose; later the same amount of insulin-gelatin was similarly administered with 0.16 mg aluminum, a weak dose. Figure 7 shows that the strong dose of aluminum inhibited the hypoglycemic effect of insulin and Fig. 8 shows reenforcement and prolongation produced by the weak aluminum dose.

Table 23

(1) ATP (μmoles)		(2) DNA-P (μmoles)	(3) 100 ATP/DNA-P	Av.	(4) % of Control
Series 1					
Control	3.19	91	3.50		100
Al	1.78	85	2.09		60
Al + PO_4	2.34	88	2.66		76
Series 2					
Control	5.05	105.6	4.78	4.86	100
	5.09	102.8	4.95		
Al	3.33	93.5	3.56	3.44	71
	2.98	89.6	3.32		
Al + PO_4	3.89	90.5	4.30	4.16	86
	3.79	94.4	4.01		

Values expressed in terms of 5.0 ml of whole blood.

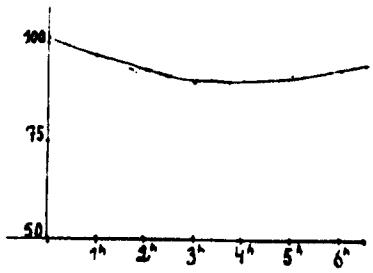

Fig. 7

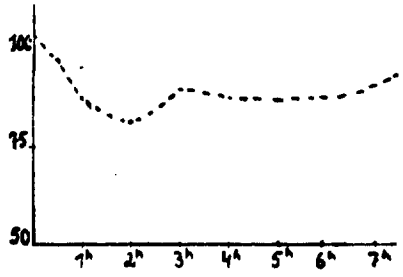

Fig. 8

In the second experiment, carried out on fasting rabbits as in the first experiment, adrenaline (0.30 mg/kg) was injected simultaneously first with 0.87 mg/kg aluminum (as the chloride) and then similarly with 0.04 mg/kg aluminum.

Figure 9 shows that with the strong aluminum dose the hyperglycemic effect of adrenaline was inhibited while Fig. 10 shows that a weak aluminum dose reenforced and prolonged the effect of adrenaline on glycemia.

The authors postulate that these unusual effects produced by the aluminum (and zinc) salt on these hormones are the result of an action on the cellular substrate in which the colloidal structure of the protoplasm is modified, which in turn alters its basic properties.

To summarize: the action of aluminum (as aluminum chloride) on the duration of the hypoglycemic effect of insulin and the hyperglycemic effect of adrenaline was found to be similarly dose dependent in both cases; weak doses strengthened and prolonged the effect and strong doses inhibited it.

D. Dogs

1. Ivy et al. (081) carried out experiments to determine the real effect of aluminum medication on gastric secretion. Two experiments were performed over 4 months with two different preparations (aluminum hydroxide cream and a colloidal aluminum hydroxide preparation, Alucol). In the experiment with the cream, 22 animals (vigorous healthy dogs) were fed the nonmedicated meal 147 times and the medicated meal 137 times. In the experiment with Alucol (28 animals), the nonmedicated meal was fed 156 times and the medicated meal 117 times.

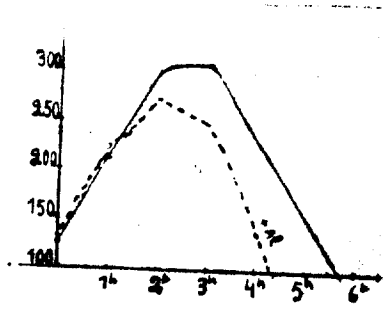

Fig. 9

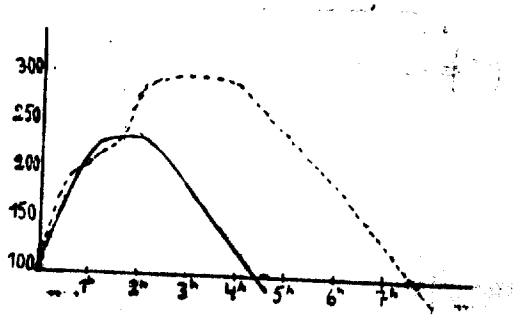

Fig. 10

It was observed that the Alucol exerted a greater "buffering" effect, which was attributed both to the larger dose administered compared to the cream and its lower degree of solubility. (See Tables in original paper.) The authors conclude that feeding these aluminum preparations over a relatively long period to normal dogs does not result in a decrease in the gastric secretory response to a meal.

2. Fauley et al. (051) showed that relatively large doses of aluminum hydroxide interfere with the absorption of phosphates in dogs. Data are presented in the paper (see original paper and Biochemical Section II) which demonstrate the effect of aluminum hydroxide on the absorption of phosphates from the intestine.

E. Humans

1. Ivy et al. (081) studied the buffering effect on gastric acid of two aluminum hydroxide preparations with human subjects. The experiments were performed with volunteer subjects (one group of 6 and another of 10). The subjects ate a meal and then varying amounts at varying frequencies (hourly and 6 times a day) of either Creamalin (an aluminum hydroxide cream) or Alucol (a colloidal aluminum hydroxide preparation). The results show that both aluminum preparations buffer free gastric acid and are more effective in their buffering action when administered more frequently.

2. Fauley et al. (051) studied the effect of aluminum hydroxide gel on phosphorus retention in humans. Four men were given a diet supplemented with calcium and phosphorus, followed by ingesting aluminum hydroxide (240 ml of a 5% aluminum hydroxide gel divided into 3 doses) daily with meals. The results (see table in original paper) showed that the ingestion of aluminum

hydroxide interfered with phosphate absorption. In a study with a 5-year-old child with marble bone disease, a condition in which there is a high degree of phosphorus retention, it was found that a high dose of aluminum hydroxide (30 ml four times a day) given in conjunction with a low phosphorus diet resulted in a negative phosphorus balance.

3. Grondahl and West (066) studied the effect of aluminum hydroxide on digestion and absorption in human subjects. Two subjects, one normal and one a hospital patient with gastric ulcer, were used. Both were given Amphojel throughout the day. The results (see tables in original paper) show that utilization of carbohydrate fats or proteins in the diet was not interfered with by aluminum hydroxide ingestion in a normal subject. The results for the gastric ulcer patient were similar except those on protein utilization were inconclusive. Slight increases in urinary pH and definite decreases in total urinary acidity were caused by aluminum hydroxide administration.

V. Drug Interaction

Humans

Wegria et al. (194) studied the effect of commercial aluminum hydroxide on the serum salicylate level. One normal subject and four patients with active rheumatic fever were given doses of from 0.9 to 1.2 g of aspirin every 4 hours (over 24 hours). When a stable serum salicylate level was established, 5 ml commercial aluminum hydroxide was given with each aspirin dose. Table 24 summarizes the results. It was found that the aluminum hydroxide as administered did not modify the serum salicylate level.

Table 24. Successive Daily Salicylate Levels (Micrograms per Cubic Centimeter of Serum)

	Subjects				
	1	2	3	4	5
Control period - aspirin alone	360, 400, 403, 404	328, 319, 317, 335	332, 326, 328	480, 475, 479, 493, 492, 478	215, 211, 210, 214, 214
Aspirin (control dose) and 5 ml aluminum hydroxide	374, 392, 400, 390, 408	351, 362, 368, 365, 412, 417, 426, 448	328, 319, 329	414, 493, 489, 477, 506	231, 191, 210, 227, 238, 220, 219
Aspirin (control dose) continued - aluminum hydroxide withdrawn	390	486, 484, 444, 480	326	477, 488, 451	239, 216, 232
Aspirin (control dose) and 2.4 g sodium bicarbonate		337, 242			
Aspirin (control dose) continued - sodium bicarbonate withdrawn		244, 354			

VI. Consumer Exposure

Of the aluminum salts that go into the processing of food, sodium aluminum phosphate is the most widely used. More than 23 million pounds were produced in the United States in 1970, as shown by an NAS/NRC survey (128) (Table 25).

Other aluminum salts are used in a wide variety of ways: sodium aluminum phosphate, in addition to being a leavening agent, is a cheese emulsifier; aluminum potassium sulfate is a firming agent used in pickling cucumbers and making maraschino cherries; in conjunction with alkaline earth, aluminum metabisulfites are used to preserve fruits and vegetables; aluminum sodium sulfate, a buffer and neutralizing agent, is used in brewing and baking powders; sodium aluminate is added to green vegetables before canning as a color preservative; aluminum sulfate is used in tanning sausage casings; aluminum compounds are used in brewing to remove organic colloids.

In making sugar, bauxite is used as an adsorbent and sodium aluminate as an auxiliary coagulant for the clarification of juices; in this capacity it helps remove some of the aluminum naturally present in cane juices. Aluminum potassium sulfate, aluminum sodium sulfate, and aluminum ammonium sulfate are buffers and neutralizing agents, the latter in baking powders. Aluminum hydroxide is an important constituent of antacid tablets and gels. These include the well-known Gelusil, Maalox, Di-Gel, and Amphogel.

The literature searched contained no reference to aluminum oleate, aluminum palmitate, or sodium phosphoaluminate used as food additives. Table 26, taken from an NAS/NRC survey (128), lists the amounts of four aluminum salts added to foods.

Table 25. Annual Poundage Data for NAS Appendix A Substances (Groups I and II)

Substance name (survey no.)	# Reports to NAS 1960/1970	Poundage reported to NAS (matching reports for both years) 1960	1970	Total 1970 poundage reported to NAS	# Reports to FEMA	Poundage reported to FEMA 1970 only	Total 1970 poundage NAS + FEMA
Aluminum Ammonium Sulfate NAS 0008	0/4	293,833	283,833	301,573	--	---	301,573
Aluminum Potassium Sulfate NAS 0009	0/4	33	950	4,950	--	---	4,950
Aluminum Sodium Sulfate NAS 0010	4/5	1,388,884	4,761,500	4,762,400	--	---	4,762,400
Aluminum Sulfate NAS 0011 FEMA 3547	10/13	513,883	663,333	666,814	--	6,666	673,480
Sodium Aluminum Phosphate NAS 0179 FEMA 3657	19/26	7,470,500	21,183,812	23,305,687	--	233	23,305,920

Table 26. Usage Levels Reported for NAS Appendix A Substances (Group I) Used in Regular Foods (R)

Substance name (Survey no.)	Food no.	Category name	# Firms reporting	- Usual use - WTD mean, %	- Maximum use - WTD mean, %
Aluminum Ammonium Sulfate NAS 0008	10	Meat prods (R)	-	.00200	.00300
	15	Condm relsh (R)	-	.09885	.25980
Aluminum Potassium Sulfate NAS 0009	15	Condm relsh (R)	-	.00084	.00119
	26	Reconst veg (R)	-	--	--
	28	Imit dairy (R)	-	--	--
Aluminum Sodium Sulfate NAS 0010	01	Baked goods (R)	-	1.08911	1.08911
	26	Reconst veg (R)	-	--	--
Aluminum Sulfate NAS 0011 FEMA 3547	01	Baked goods (R)	-	.16000	.24500
	14	Procsd vegs (R)	5	.00507	.00647
	15	Condm relsh (R)	5	.23185	.31568
	20	Gelatin pud (R)	-	.01000	.10000
	48	Sea flavrs (R)	-	1.00000	1.00000
Sodium Aluminum Phosphate NAS 0179 FEMA 3657	01	Baked goods (R)	23	1.19135	1.56896
	03	Other grain (R)	-	.35828	.41925
	06	Cheese (R)	-	2.21736	2.40518
	10	Meat prods (R)	-	.13580	.13580

Aluminum as a metal occurs naturally in many foods, and Table 27 (from Truffert, 181) shows the amounts of aluminum found in a variety of staples. As pointed out in Chemical Section VIII, A, the amount of aluminum found in plants is related to local soil and atmospheric conditions. Plants, as this table shows, consequently have a higher aluminum content than most animal products. The higher content in canned fish and meat shown in Table 27 is quite possibly due to preparation or storage in aluminum containers. A more extensive table showing amounts of aluminum occurring in foods can be found in Campbell et al. (Table 6, 25).

The amount of aluminum or aluminum salts reaching the consumer will vary according to his location and diet. One author (Vozar, Voprosy Pitaniya 1962(3):28, quoted in 25) estimates the concentration of aluminum in the ordinary mixed diet of an adult as 80.5 mg, with the amount doubling after heat processing in aluminum kettles. Campbell et al. (25) collated a variety of estimates of daily intakes of aluminum by man, reproduced here in Table 28. The possible daily intake of specific aluminum salts, as estimated by the NAS/NRC (128) is shown in Table 29. As would be expected, the intake of sodium aluminum phosphate from baked foods is rather high--1640 mg per day average intake. Aluminum sodium sulfate is next highest, with 1494 mg average daily intake.

The use of aluminum in cooking utensils has been controversial since the discovery of the metal. The resistance of aluminum to corrosion is owing to a protective film on the surface of the metal; when this film is broken down by acids or salts, particularly heavy metal salts, the Al is corroded. Cold fruit juices have little effect on aluminum, but boiling citrus juices attack it severely. The dilution of juices also affects

Table 27

Food substances	Aluminum content (mg/kg)
1. Products of butchery, pork butchery, tripe shop, poultry, game	20 (fresh) and 100 (canned)
2. Fish	20 (fresh) and 100 (canned)
3. Mollusks, crustaceans	29 (fresh) and 100 (canned)
4. Eggs	50
5. Oils and fats	50
6. Milk[a]	50
7. Wine, beer, cider, fruit juice and other alcholic or other beverages	50
8. Vegetables and fruits[b]	250
9. Cereals and derived products[b]	200
10. Cheeses[b]	200
11. Sugars, sweetening substances, jams[b]	200

[a] Dose expressed per liter.
[b] Content reported moisture free.

Table 28. Estimates of Daily Intake of Aluminum by Man[a]

Date	Author	Daily Aluminum Intake, mg: From natural diet; no known contact with Al	Daily Aluminum Intake, mg: From natural diet and contact with Al utensils and/or use of Al baking powder	
1914	U.S. Dept. of Agriculture (based on Ref. Bd. findings; Al ingested as baking powder)		25-75 in usual diet 150-200 extreme conditions	baking powder only
1926	Jacobs (estimate based on early high values in raw products; calculated Al content in meals over a period of 7 days)	472 (av.)		
1929	Lehmann (estimate based on own experiments)	20-25	40-60 100 maximum	including acid foods cooked in aluminum
1931	Fellenberg (estimate based on own experiments)	5-10	5-10+8-10	from natural sources + from aluminum utensils
1931	Eppinger (estimate based on literature)		50	
1932	Burn (estimate based on Massatsch's values)		7	
1932	Beal (estimate based on own experiments)	7 (av.)	12	(if all foods are cooked in Al utensils)
1935	Datta (estimate based on own experiments)		<50	(under conditions found in India)
1940	Kehoe et al. (analysis of total intake of food and beverages of 1 male adult for 28 days)		36.43 ± S.D. 61.97 mean values	
1942	Calvery (estimate based on literature)		10-30 ppm	
1943	Kehoe et al. (analysis of mean intake of food and beverages of 1 male adult (other than above) for 15 wk.)		17.31 ± S.D. 27.67 mean values	

Table 28 (Continued)

Date	Author	Daily Aluminum Intake, mg: From natural diet; no known contact with Al	Daily Aluminum Intake, mg: From natural diet and contact with Al utensils and/or use of Al baking powder	
1947	Hadorn (estimate based on own experiments)	1.5-10; 6 in 1660 g food	1.5-10+0.1-8	from natural sources (maximum includes 2 meals with acid foods)(in 250 g baked goods) baking powder only
1955	Editorial (estimate based on 1.5 kg daily food consumption; review of literature)		115 8-10 135 maximum	+ natural content (if all foods were cooked with soda)

[a] See original paper for references.

Table 29. Possible Daily Intakes of NAS Appendix A Substances (Groups I and II), per Food Category and Total Dietary, Based on Food Consumption by Total Sample -- See Explanatory Notes in Exhibits Section

Substance name (survey no.)	Food no.	Category name	# of Firms	Age	Possible daily intake, mg Average	High A	High B
Aluminum Ammonium Sulfate NAS 0008	10	Meat prods (R)	-	0- 5 mo.	.022000	.058000	.033000
				6-11 mo.	.414000	1.116000	.621000
				12-23 mo.	.604000	1.038000	.906000
				2-65+ yr.	1.568000	2.602000	2.352000
Aluminum Ammonium Sulfate NAS 0008	15	Condm relsh (R)	-	0- 5 mo.	---	.098850	---
				6-11 mo.	.790800	2.174700	2.078400
				12-23 mo.	2.767800	7.512600	7.274400
				2-65+ yr.	8.698800	20.956200	22.862400
Aluminum Ammonium Sulfate NAS 0008	All categories		-	0- 5 mo.	.022000	.156850	.033000
				6-11 mo.	1.204800	3.290700	2.699400
				12-23 mo.	3.371900	8.550600	8.180400
				2-65+ yr.	10.266800	23.558200	25.214400
Aluminum Potassium Sulfate NAS 0009	15	Condm relsh (R)	-	0- 5 mo.	---	.000840	---
				6-11 mo.	.006720	.018480	.009520
				12-23 mo.	.023520	.063840	.033320
				2-65+ yr.	.073920	.178080	.104720
Aluminum Potassium Sulfate NAS 0009	26	Reconst veg (R)	-	0- 5 mo.	---	---	---
				6-11 mo.	---	---	---
				12-23 mo.	---	---	---
				2-65+ yr.	---	---	---
Aluminum Potassium Sulfate NAS 0009	28	Imit dairy (R)	-	0- 5 mo.	---	---	---
				6-11 mo.	---	---	---
				12-23 mo.	---	---	---
				2-65+ yr.	---	---	---
Aluminum Potassium Sulfate NAS 0009	All categories		4	0- 5 mo.	---	.000840	---
				6-11 mo.	.006720	.018480	.009520
				12-23 mo.	.023520	.063840	.033320
				2-65+ yr.	.073920	.178080	.104720

Table 29 (Continued)

Substance name (survey no.)	Food no.	Category name	# of Firms	Age	Possible daily intake, mg Average	High A	High B
Aluminum Sodium Sulfate NAS 0010	01	Baked goods (R)	-	0- 5 mo.	37.029740	49.009950	37.029740
				6-11 mo.	276.633940	564.158980	276.633940
				12-23 mo.	593.564950	978.020780	593.564950
				2-65+ yr.	1494.258920	2219.606180	1494.258920
Aluminum Sodium Sulfate NAS 0010	26	Reconst veg (R)	-	0- 5 mo.	-----	-----	-----
				6-11 mo.	-----	-----	-----
				12-23 mo.	-----	-----	-----
				2-65+ yr.	-----	-----	-----
Aluminum Sodium Sulfate NAS 0010	All categories		4	0- 5 mo.	37.029740	49.009950	37.029740
				6-11 mo.	276.633940	564.158980	276.633940
				12-23 mo.	593.564950	978.020780	593.564950
				2-65+ yr.	1494.258920	2219.606180	1494.258920
Aluminum Sulfate NAS 0011	01	Baked goods (R)	-	0- 5 mo.	5.440000	7.200000	8.330000
				6-11 mo.	40.640000	82.880000	62.230000
				12-23 mo.	87.200000	143.680000	133.525000
				2-65+ yr.	219.520000	326.080000	336.140000
Aluminum Sulfate NAS 0011	14	Procsd vegs (R)	5	0- 5 mo.	.070980	.212940	.090580
				6-11 mo.	1.216800	2.839200	1.552800
				12-23 mo.	1.977300	3.310710	2.523300
				2-65+ yr.	4.309500	7.260240	5.499500
Aluminum Sulfate NAS 0011	15	Condm relsh (R)	5	0- 5 mo.	-----	.231850	-----
				6-11 mo.	1.854800	5.100700	2.525440
				12-23 mo.	6.491800	17.620600	8.839040
				2-65+ yr.	20.402800	49.152200	27.779840
Aluminum Sulfate NAS 0011	20	Gelatin pud (R)	-	0- 5 mo.	.200000	.270000	2.000000
				6-11 mo.	1.280000	3.880000	12.800000
				12-23 mo.	1.380000	3.360000	13.800000
				2-65+ yr.	2.040000	5.250000	20.400000

Table 29 (continued)

Substance name (survey no.)	Food no.	Category name	# of Firms	Age	Possible daily intake, mg Average	High A	High B
Aluminum Sulfate NAS 0011	48	Seas flavrs (R)	-	0- 5 mo.	-----	-----	-----
				6-11 mo.	-----	.100000	-----
				12-23 mo.	-----	.200000	-----
				2-65+ yr.	.100000	.500000	.100000
Aluminum Sulfate NAS 0011	All categories		14	0- 5 mo.	5.710980	7.914790	10.420580
				6-11 mo.	44.991600	94.799900	79.108240
				12-23 mo.	97.049100	168.171310	158.687340
				2-65+ yr.	246.372300	388.242440	389.919340
Sodium Aluminum Phosphate NAS 0179	01	Baked goods (R)	23	0- 5 mo.	40.505900	53.610750	53.344640
				6-11 mo.	302.602900	617.119300	398.515840
				12-23 mo.	649.285750	1069.832300	855.083200
				2-65+ yr.	1639.532200	2427.971300	2152.613120
Sodium Aluminum Phosphate NAS 0179	03	Other grain (R)	-	0- 5 mo.	1.791400	6.090760	2.096250
				6-11 mo.	34.753160	102.468080	40.667250
				12-23 mo.	58.757920	135.788120	68.757000
				2-65+ yr.	99.601840	219.983920	116.551500
Sodium Aluminum Phosphate NAS 0179	06	Cheese (R)	-	0- 5 mo.	-----	2.217360	-----
				6-11 mo.	59.868720	215.083920	64.939860
				12-23 mo.	172.954080	492.253920	187.604040
				2-65+ yr.	208.431840	523.296960	226.086920
Sodium Aluminum Phosphate NAS 0179	10	Meat prods (R)	-	0- 5 mo.	1.493800	3.938200	1.493800
				6-11 mo.	28.110600	75.776400	28.110600
				12-23 mo.	41.011600	70.480200	41.011600
				2-65+ yr.	106.467200	176.675800	106.467200
Sodium Aluminum Phosphate NAS 0179	All categories		27	0- 5 mo.	43.791100	65.857070	56.934690
				6-11 mo.	425.335380	1010.447700	532.233550
				12-23 mo.	922.009350	1768.354540	1152.455840
				2-65+ yr.	2049.033080	3347.927980	2601.718740

their corrosiveness, more dilute juices reacting more strongly with Al (025). Salt enhances corrosion while sugar inhibits it. Vegetables have a negligible effect on Al vessels (025). Aluminum appears to be practically free from attack by meats and fatty acids. Fresh milk has no effect on Al, but strongly soured milk or buttermilk and sour rennet whey attack aluminum (025).

ALUMINUM COMPOUNDS

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March 13, 1970

I hereby acknowledge receipt, this day, of the following numbered exhibits from Dr. Harris L. Coulter, representing the National Health Federation: 58-1 to 58-56, 58-58 to 58-68, 58-70, 58-100 to 58-105, 58-107, 58-108, 58-120, 58-122, 58-131, 58-132, 58-151 to 58-155, 58-175 to 58-177, 58-180 to 58-186, 58-204, o-7-58, o-76-58, O-147-58, O-149-58, O-151-58. O-153-58, O-155-58, O-175-58 O-190-58 O-193-58, O-194-58, O-209-58, O-329-58, O-379-58.

Beryl McCullar
Hearing Clerk
Parklawn Building
Rockville, Md.

The above checked numbered exhibits were received in the Office of the Hearing Clerk, but note the following exceptions:

No 58-27 Exhibit marked 202-45

No 104 - Note in file- Found in O-329-58

No 58-176 - Note in file-Identical to 58-100

No 58-177 " " " - " " 58-107

Food and Drug Research Laboratories
INCORPORATED

Waverly Division
Route 17
P.O. Box 107
Waverly, New York 14892

Maurice Avenue at 58th Street
Maspeth, New York 11378
Telephone: TWining 4-0800
Cable: Foodlabs, New York

August 31, 1972

COPY

Mr. L. C. Appleby, Contracts Officer
Department of Health, Education and Welfare
Food and Drug Administration
Contracts Section CA-272
Contracts and Grants Branch
5600 Fishers Lane
Rockville, Maryland 20852

Subject: Teratologic Studies, Final Reports (Three species)
Re: FDA Contract No. 71-260

Dear Mr. Appleby:

We are today forwarding final reports covering teratologic studies on FDA Compounds 71-18 and 71-42 as follows:

Your office	1 copy (via Mr. Carle's office)
Dr. Alan Spiher	2 copies
Dr. Joseph McLaughlin	2 copies

These final copies correspond to drafts dated July 31, 1972.

If you have any further instructions, or questions, please do not hesitate to contact us.

Cordially,

FOOD and DRUG RESEARCH LABORATORIES, INC.

Kenneth Morgareidge

Kenneth Morgareidge, Ph. D.
Vice President

KM:d
cc: Dr. Alan Spiher
Dr. Joseph McLaughlin
Mr. D. A. Carle

PROJECT NO: BTL-71-49
REPORT FILE

Industrial BIO-TEST Laboratories, Inc.
1810 FRONTAGE ROAD
NORTHBROOK, ILLINOIS 60062

1.

REPORT TO

MONSANTO COMPANY

90-DAY SUBACUTE ORAL TOXICITY STUDY WITH LEVN-LITE IN BEAGLE DOGS

BTL-71-49

JUNE 21, 1972

IBT NO. J749

Sodium Aluminum Phosphate — (Acidic)

Industrial **BIO-TEST** *Laboratories, Inc.*
1810 FRONTAGE ROAD
NORTHBROOK, ILLINOIS 60062

June 21, 1972

Dr. George J. Levinskas
Manager, Product Evaluation
Monsanto Company
800 North Lindbergh Boulevard
St. Louis, Missouri 63166

Dear Dr. Levinskas:

Re: IBT No. J749 - 90-Day Subacute Oral Toxicity
Study with Levn-Lite in Beagle Dogs - BTL-71-49

We are submitting herewith our laboratory report dated June 21, 1972, prepared in connection with the above study.

Very truly yours,

J. C. Calandra
President

JCC/kjl

REPORT TO

MONSANTO COMPANY

90-DAY SUBACUTE ORAL TOXICITY STUDY WITH LEVN-LITE IN BEAGLE DOGS

BTL-71-49

JUNE 21, 1972

IBT NO. J749

I. Introduction

A sample identified as Levn-Lite was received from Monsanto Company for the purpose of conducting a 90-day subacute oral toxicity study using purebred beagle dogs. The following report presents the results of the investigation.

II. Summary

Ninety-day oral administration of Levn-Lite to purebred beagle dogs at dietary levels of 0.3, 1.0 and 3.0 percent revealed no significant abnormalities in the following parameters:

Body Weights	Blood Chemistry Studies
Food Consumption	Organ Weights
Behavioral Reactions	Urine Analyses
Mortality	Gross Pathologic Studies
Hematologic Studies	Histopathologic Studies

Respectfully submitted,

INDUSTRIAL BIO-TEST LABORATORIES, INC.

Report prepared by:

Kenneth Mastalski, B.S.
Group Leader
Wedge's Creek Research Farm

Report approved by:

Donald H. Jenkins, D.V.M.
Manager & Technical Director
Wedge's Creek Research Farm

M. L. Keplinger, Ph.D.
Manager, Toxicology

lk:sjn:psh

III. Procedure

A. Organization

The 90-day toxicity study utilized an untreated control group and three test groups, each consisting of eight purebred beagle dogs (four males and four females). The beagles were all eligible for A.K.C. registration and had been previously immunized against rabies, distemper, infectious canine hepatitis and leptospirosis.

All dogs were acquired form our own (IBTL) colony and were under observation for two weeks prior to the start of the investigation, during which time they were reimmunized and rendered clinically free of any existing parasitic infestation.

During the investigation, the selected animals were housed in kennels equipped with outside runs, four dogs of the same sex and group being accommodated in a single kennel.

The material to be tested, Levn-Lite, was incorporated into a stock diet and fed to the dogs seven days a week at three graded dietary levels. The levels were 0.3, 1.0 and 3.0 percent of test material in the diet.

An outline of the test organization and diet composition is presented in Table I.

TABLE I

TEST MATERIAL: Levn-Lite

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Test Organization and Diet Composition

Group	Number of Animals		Constituents of Diet	
	Male	Female	Dose Level (%)	Stock Ration* (%)
UC	4	4	None	100.0
T-I	4	4	0.3	99.7
T-II	4	4	1.0	99.0
T-III	4	4	3.0	97.0

* Golden Choice Meals, Adolph Coors Company, Denver, Colorado.

B. Parameters Investigated

Initially, the body weight of each dog in every group was determined and recorded. Thereafter, weighings were conducted weekly for the duration of the test.

At the beginning of each week, the appropriate dietary constituents for each of the groups were thoroughly blended in a Hobart mixer. Preweighed amounts were distributed into self-feeding units and maintained in excess of the animals' consumption. One such unit was available to the dogs in each kennel on an ad libitum basis 24 hours a day. At the end of each seven-day period, all unconsumed food was collected and weighed. Food consumption was then calculated and recorded. Water was available to the animals at all times.

The dogs were under observation during the investigation and were examined daily for clinical signs or symptoms indicative of systemic toxicity.

The following determinations were conducted upon each dog from the untreated control group and three test groups just prior to the inception of the study and after 42 and 84 days of testing:

Hematologic Studies

total leukocyte count
erythrocyte count
hemoglobin
hematocrit
differential leukocyte count

Blood Chemistry Studies

blood urea nitrogen
serum glucose
serum alkaline phosphatase
serum glutamic-oxalacetic transaminase
serum glutamic-pyruvic transaminase

Urine Analyses

albumin
glucose
pH
microscopic elements - leukocytes
erythrocytes
crystals

At the conclusion of the investigation, the dogs from each group were sacrificed by electric shock. All major tissues and organs were examined grossly. The weights of the following organs were obtained: liver, kidneys, heart, brain, spleen, gonads, adrenal glands, thyroid gland and pituitary gland. The following tissues and organs excised from these animals were examined histologically (Hematoxylin-Eosin Stain):

Adrenal Glands
Aorta (thoracic)
Bone Marrow (sternum)
Brain (cerebrum, cerebellum, pons)
Caecum
Colon
Esophagus
Gall Bladder
Gonads
Heart
Kidneys
Liver
Lymph Nodes (cervical, mesenteric)
Muscle (skeletal)
LUNGS
Pancreas
Peripheral Nerve (sciatic)
Pituitary Gland
Prostate Gland
Salivary Gland (submaxillary)
Small intestine (duodenum, jejunum, ileum)
Spinal Cord
Spleen
Stomach (cardia, fundus, pylorus)
Trachea
Thyroid Gland
Urinary Bladder

IV. Results

A. Body Weight Data

The body weight data are presented in Tables II and III.

No significant deviations from normally expected body weight gains for dogs of this age were noted.

TABLE II

TEST MATERIAL: Levn-Lite

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Body Weight Data for Males, kilograms

Group	Dietary Level (%)	Dog Number	Age at Inception of Test (months)	Body Weights at Week Indicated: 0	1	2	3	4	5	6	7	8	9	10	11	12	13	Overall Weight Gain
UC	None	1	5.5	10.0	10.1	10.1	9.8	10.3	10.0	11.0	10.8	10.6	10.8	11.1	11.1	10.9	11.0	1.0
		2	6.0	9.5	9.7	9.7	10.3	10.5	10.4	11.1	11 3	11.4	11.7	12.1	12.1	12.2	11.9	2.4
		3	6.0	10.2	10.1	10.5	10.8	10.2	10.6	11.5	11.8	11.7	12.2	12.5	12.3	11.9	12.3	2.1
		4	6.0	8.4	8.5	8.6	8.6	8.9	8.5	9.3	9.5	9.5	9.8	10.2	10.0	9.9	10.2	1.8
		Mean	5.9	9.5	9.6	9.7	9.9	10.2	10.1	10.7	10.8	10.8	11.1	11.5	11.4	11.2	11.4	1.9
T-I	0.3	9	5.5	11.0	11.1	11.5	11.7	11.8	11.9	12.2	12.4	12.3	12.6	12.6	12.6	12.5	12.7	1.7
		10	6.0	8.6	8.8	9.2	9.2	8.9	9.4	10.3	10.1	10.2	10.6	10.7	10.9	10.7	10.9	2.3
		11	6.0	7.5	7.6	8.4	8.8	8.5	8.1	9.0	9.0	9.0	9.3	9.4	9.5	9.4	9.4	1.9
		12	6.0	8.5	8.6	9.1	9.3	9.6	9.5	10.5	10.5	10.6	11.0	11.5	11.9	11.6	11.7	3.2
		Mean	5.9	8.9	9.0	9.6	9.8	9.7	9.7	10.5	10.5	10.5	10.9	11.0	11.2	11.0	11.2	2.3
T-II	1.0	17	6.0	6.7	6.7	7.2	7.2	7.5	7.6	8.0	8.5	8.6	9.4	9.6	9.7	9.8	10.0	3.3
		18	6.0	10.1	10.2	10.6	10.3	10.8	10.7	11.9	12.0	12.0	12.1	12.5	12.7	12.5	12.4	2.3
		19	6.0	8.8	8.6	8.8	9.0	9.6	9.5	10.6	10.7	10.6	11.2	11.4	11.5	11.1	11.5	2.7
		20	6.0	9.0	9.0	9.7	9.9	10.1	9.9	10.9	10.7	11.0	11.1	11.0	11.5	11.3	11.4	2.4
		Mean	6.0	8.6	8.6	9.1	9.1	9.5	9.4	10.4	10.5	10.6	11.0	11.1	11.4	11.2	11.3	2.7
T-III	3.0	25	6.0	5.9	6.1	6.4	6.4	6.8	6.6	7.6	7.5	7.6	7.7	8.1	8.2	8.3	8.3	2.4
		26	6.0	8.5	8.6	8.7	8.4	9.1	8.6	9.6	9.3	9.4	9.2	9.6	9.0	9.2	9.4	0.9
		27	6.0	10.2	10.4	10.4	10.4	11.1	10.8	12.4	12.2	12.2	12.5	12.6	12.8	12.9	12.5	2.3
		28	6.0	10.0	10.0	10.6	10.5	11.4	11.4	12.6	12.8	13.0	13.3	13.8	14.0	13.9	14.1	4.1
		Mean	6.0	8.6	8.8	9.0	8.9	9.6	9.4	10.6	10.4	10.6	10.7	11.0	11.0	11.1	11.1	2.5

TABLE III

TEST MATERIAL: Levn-Lite

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Body Weight Data for Females, kilograms

Group	Dietary Level (%)	Dog Number	Age at Inception of Test (months)	Body Weights at Week Indicated: 0	1	2	3	4	5	6	7	8	9	10	11	12	13	Overall Weight Gain
UC	None	5	6.0	6.6	6.9	7.1	7.3	7.8	7.4	7.8	7.8	7.8	8.0	8.1	8.2	8.0	8.1	1.5
		6	6.0	7.9	8.1	8.4	8.3	8.4	8.1	9.1	8.9	8.9	9.1	9.1	9.3	9.2	9.2	1.3
		7	6.0	8.0	8.4	8.8	8.7	9.0	9.0	9.7	10.0	9.7	10.2	10.1	10.6	10.5	10.5	2.5
		8	6.0	6.7	6.6	6.9	7.1	7.4	7.1	7.7	7.8	7.8	8.0	8.2	8.2	8.0	8.0	1.3
		Mean	6.0	7.3	7.5	7.8	7.8	8.0	7.9	8.6	8.6	8.6	8.8	8.9	9.1	8.9	9.0	1.7
T-I	0.3	13	5.5	6.0	6.4	6.6	6.6	6.5	6.2	7.3	7.1	7.2	7.2	7.3	7.4	7.1	7.3	1.3
		14	5.5	4.8	5.0	5.2	5.2	5.2	5.4	5.7	5.7	5.7	5.7	5.8	5.8	5.7	5.7	0.9
		15	6.0	9.8	10.2	10.8	10.7	10.9	11.1	11.6	11.6	11.7	11.7	11.7	11.7	11.7	11.7	1.9
		16	6.0	8.3	9.0	9.4	9.6	9.3	9.6	10.3	10.0	10.2	10.4	10.5	10.6	10.6	10.6	2.3
		Mean	5.8	7.2	7.6	8.0	8.0	8.0	8.1	8.7	8.6	8.7	8.8	8.8	8.9	8.8	8.8	1.6
T-II	1.0	21	5.5	5.7	5.8	6.2	6.0	6.4	6.0	6.2	6.8	6.9	7.0	6.9	7.1	7.1	7.1	1.4
		22	5.5	7.5	7.4	7.9	7.7	8.0	7.6	8.3	8.4	8.3	8.4	8.5	8.7	8.7	8.7	1.2
		23	5.5	4.3	4.5	4.7	4.5	4.8	4.4	5.1	5.1	5.3	5.3	5.3	5.9	5.5	5.5	1.2
		24	6.0	8.1	8.1	8.5	8.4	8.9	8.8	9.2	9.5	9.4	9.6	9.8	9.7	9.7	9.7	1.6
		Mean	5.6	6.4	6.4	6.8	6.6	7.0	6.7	7.2	7.4	7.5	7.6	7.6	8.0	7.8	7.8	1.4
T-III	3.0	29	5.5	5.1	5.1	5.1	5.1	5.2	4.8	5.8	5.7	5.7	5.7	5.8	5.9	5.9	5.9	0.8
		30	6.0	7.5	7.8	8.3	8.5	9.0	8.8	10.0	10.1	10.2	10.8	11.0	11.4	11.4	11.4	3.9
		31	6.0	7.3	7.3	7.4	7.6	7.9	7.5	8.5	8.4	8.4	8.7	8.9	9.0	9.1	8.9	1.6
		32	6.0	7.0	7.0	7.3	7.3	7.4	8.0	8.1	8.1	8.2	8.4	8.6	8.9	8.5	8.6	1.6
		Mean	5.9	6.7	6.8	7.0	7.1	7.4	7.3	8.1	8.1	8.1	8.4	8.6	8.8	8.7	8.7	2.0

B. Food Consumption Data

Food consumption data are presented in Table IV.

There is no significant difference between the untreated control group and the three test groups.

TABLE IV

TEST MATERIAL: Levn-Lite

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Food Consumption Data

		Mean Food Consumed During Week Indicated (grams/day)							
	Sex:	Males				Females			
	Group:	UC	T-I	T-II	T-III	UC	T-I	T-II	T-III
Week	Dietary Level (%):	None	0.3	1.0	3.0	None	0.3	1.0	3.0
1		352	333	375	358	380	395	371	412
2		361	364	404	406	417	419	415	439
3		366	347	404	407	397	378	364	405
4		338	340	384	393	366	316	407	412
5		356	348	380	378	399	336	396	414
6		348	336	370	387	375	362	375	395
7		319	328	310	336	343	302	399	355
8		285	310	340	321	323	307	320	389
9		315	307	298	287	321	287	355	366
10		333	341	349	341	362	338	401	410
11		300	304	320	327	341	309	331	346
12		286	282	286	286	298	270	352	333
13		281	266	299	278	285	292	299	268
Mean		326	324	348	346	354	332	368	380

C. Reactions

No untoward behavioral reactions were recorded during the investigation.

D. Mortality

No fatalities occurred during the investigation.

E. Hematologic Studies

The results of these determinations are presented in Tables V through IX.

No significant abnormalities were noted at any levels tested.

TABLE V

TEST MATERIAL: Levn-Lite

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Hematologic Studies: Total Leukocyte Count, thousands/mm^3

Group	Dietary Level (%)	Dog Number and Sex	Days: 0	42	84
UC	None	1-M	14.2	12.3	9.3
		2-M	19.9	17.2	14.7
		3-M	17.0	18.0	15.4
		4-M	16.4	15.2	10.7
		5-F	12.9	14.6	11.1
		6-F	12.2	14.0	11.9
		7-F	14.0	15.2	11.9
		8-F	9.9	12.7	12.7
T-I	0.3	9-M	15.3	14.6	11.5
		10-M	12.8	14.1	11.5
		11-M	28.8	18.2	13.2
		12-M	14.2	13.2	10.7
		13-F	11.3	11.3	9.1
		14-F	15.7	14.2	13.6
		15-F	14.9	11.5	9.9
		16-F	12.7	12.6	11.2
T-II	1.0	17-M	12.7	12.2	10.8
		18-M	12.4	11.5	10.7
		19-M	15.3	14.5	10.1
		20-M	20.5	17.4	13.1
		21-F	11.3	16.3	11.5
		22-F	14.6	12.0	10.1
		23-F	18.3	16.1	12.3
		24-F	10.9	12.6	9.6
T-III	3.0	25-M	11.9	10.5	8.2
		26-M	13.3	14.3	11.1
		27-M	17.2	14.9	12.9
		28-M	13.2	13.1	10.8
		29-F	13.8	15.6	12.8
		30-F	12.7	12.3	10.9
		31-F	12.6	11.3	9.5
		32-F	13.0	16.5	12.0

TABLE VI

TEST MATERIAL: Levn-Lite

90-Day Subacute Oral Toxicity Study - Beage Dogs

Hematologic Studies: Erythrocyte Count, millions/mm^3

Group	Dietary Level (%)	Dog Number and Sex	Days: 0	42	84
UC	None	1-M	6.57	6.64	7.56
		2-M	7.06	6.60	6.89
		3-M	6.45	6.69	6.79
		4-M	5.68	6.06	6.37
		5-F	6.58	6.57	7.09
		6-F	6.50	6.98	6.92
		7-F	6.48	7.10	6.98
		8-F	5.89	6.48	6.76
T-I	0.3	9-M	6.93	6.97	7.12
		10-M	5.34	5.97	6.77
		11-M	6.52	6.28	7.27
		12-M	5.87	6.03	6.85
		13-F	5.66	6.36	6.48
		14-F	6.03	6.47	6.67
		15-F	7.04	6.97	7.85
		16-F	6.37	6.50	7.38
T-II	1.0	17-M	4.74	4.98	5.48
		18-M	5.84	6.08	6.13
		19-M	5.91	6.52	6.95
		20-M	6.16	6.55	6.87
		21-F	6.05	6.06	6.57
		22-F	6.57	6.35	7.32
		23-F	5.74	5.07	6.04
		24-F	6.48	6.91	7.41
T-III	3.0	25-M	4.89	4.85	5.80
		26-M	5.41	5.37	5.62
		27-M	5.86	6.13	7.13
		28-M	5.45	5.41	6.34
		29-F	5.96	5.95	6.53
		30-F	5.61	5.75	6.67
		31-F	5.88	5.83	6.49
		32-F	6.01	6.21	6.67

TABLE VII

TEST MATERIAL: Levn-Lite

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Hematologic Studies: Hemoglobin, gm/100 ml

Group	Dietary Level (%)	Dog Number and Sex	Days: 0	42	84
UC	None	1-M	15.4	15.5	16.8
		2-M	16.3	15.7	16.0
		3-M	15.5	16.6	16.3
		4-M	14.2	14.8	15.7
		5-F	14.5	15.4	16.6
		6-F	15.3	16.8	16.1
		7-F	15.2	17.3	16.6
		8-F	14.1	15.7	16.1
T-I	0.3	9-M	15.3	16.4	16.7
		10-M	13.1	14.5	16.5
		11-M	15.1	14.8	16.9
		12-M	13.5	13.7	15.2
		13-F	13.1	15.2	14.9
		14-F	14.7	15.8	16.2
		15-F	15.9	16.4	18.6
		16-F	15.1	15.9	17.7
T-II	1.0	17-M	11.1	12.0	13.3
		18-M	13.1	14.3	14.2
		19-M	13.5	15.3	16.2
		20-M	14.1	15.0	15.5
		21-F	14.1	14.1	15.1
		22-F	15.0	15.0	16.8
		23-F	14.2	12.8	15.0
		24-F	15.8	16.3	17.7
T-III	3.0	25-M	11.0	12.3	13.8
		26-M	13.3	13.0	13.3
		27-M	13.4	15.5	17.4
		28-M	13.6	13.5	15.9
		29-F	15.1	15.1	16.4
		30-F	13.1	14.1	15.8
		31-F	13.8	13.9	15.2
		32-F	13.9	15.0	15.8

TABLE XI

TEST MATERIAL: Levn-Lite

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Blood Chemistry Studies: Serum Glucose, mg/100 ml

Group	Dietary Level (%)	Dog Number and Sex	Days: 0	42	84
UC	None	1-M	90	112	105
		2-M	127	125	108
		3-M	118	125	113
		4-M	115	103	97
		5-F	104	113	104
		6-F	121	123	99
		7-F	97	103	102
		8-F	93	108	96
T-I	0.3	9-M	111	120	105
		10-M	97	115	113
		11-M	88	103	102
		12-M	104	112	110
		13-F	84	95	87
		14-F	93	97	108
		15-F	115	120	113
		16-F	112	123	107
T-II	1.0	17-M	109	113	101
		18-M	99	105	97
		19-M	97	117	101
		20-M	104	115	105
		21-F	97	100	96
		22-F	106	109	99
		23-F	69	93	92
		24-F	101	120	114
T-III	3.0	25-M	90	100	102
		26-M	101	106	99
		27-M	71	106	111
		28-M	95	100	101
		29-F	95	110	99
		30-F	92	105	89
		31-F	107	112	102
		32-F	95	103	94

Note: colorimetric method by Roeckelt and Gochman.

TABLE XII

TEST MATERIAL: Levn-Lite

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Blood Chemistry Studies: Serum Alkaline Phosphatase, King-Armstrong Units/100 ml

Group	Dietary Level (%)	Dog Number and Sex	Days: 0	42	84
UC	None	1-M	7.06	6.63	5.23
		2-M	6.87	6.34	5.23
		3-M	6.68	6.78	5.06
		4-M	17.33	25.62	13.82
		5-F	9.45	9.10	7.36
		6-F	7.83	8.14	6.81
		7-F	7.44	7.52	6.10
		8-F	4.51	5.23	3.74
T-I	0.3	9-M	7.25	9.77	6.45
		10-M	9.45	10.29	9.48
		11-M	8.23	7.83	6.81
		12-M	6.68	6.63	5.40
		13-F	5.94	6.63	5.06
		14-F	6.30	6.92	7.55
		15-F	9.04	10.29	8.88
		16-F	6.68	6.63	5.58
T-II	1.0	17-M	6.68	6.92	8.30
		18-M	7.64	7.52	6.45
		19-M	9.04	9.43	6.99
		20-M	8.23	8.45	6.10
		21-F	6.49	6.34	5.40
		22-F	6.30	6.06	5.06
		23-F	8.23	8.30	6.45
		24-F	8.23	9.10	7.92
T-III	3.0	25-M	5.57	4.69	4.39
		26-M	5.57	5.64	7.92
		27-M	9.45	9.60	9.48
		28-M	8.23	8.45	7.92
		29-F	6.68	6.06	5.58
		30-F	10.29	10.47	9.28
		31-F	8.63	10.12	7.92
		32-F	11.61	9.77	8.30

Note: colorimetric method by Babson.

TABLE XIII

TEST MATERIAL: Levn-Lite

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Blood Chemistry Studies: Serum Glutamic-Oxalacetic Transaminase, Dade Units/ml

Group	Dietary Level (%)	Dog Number and Sex	Days: 0	42	84
UC	None	1-M	17	16	18
		2-M	24	21	21
		3-M	24	17	19
		4-M	39	20	27
		5-F	32	20	20
		6-F	14	20	18
		7-F	27	22	15
		8-F	14	15	14
T-I	0.3	9-M	22	26	24
		10-M	24	32	31
		11-M	19	20	19
		12-M	30	26	20
		13-F	19	22	18
		14-F	27	23	24
		15-F	21	18	22
		16-F	17	20	18
T-II	1.0	17-M	26	15	13
		18-M	17	19	15
		19-M	31	30	19
		20-M	21	25	33
		21-F	16	19	14
		22-F	16	18	14
		23-F	27	19	19
		24-F	27	27	20
T-III	3.0	25-M	30	20	20
		26-M	27	19	16
		27-M	37	17	22
		28-M	29	23	19
		29-F	22	18	20
		30-F	22	23	21
		31-F	17	22	19
		32-F	33	29	25

Note: fluorometric method by Levine and Hill.

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TABLE XIV

TEST MATERIAL: Levn-Lite

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Blood Chemistry Studies: Serum Glutamic-Pyruvic Transaminase, Dade Units/ml

Group	Dietary Level (%)	Dog Number and Sex	Days: 0	42	84
UC	None	1-M	15	15	17
		2-M	66	19	27
		3-M	15	16	24
		4-M	15	15	27
		5-F	15	14	15
		6-F	15	15	16
		7-F	15	15	16
		8-F	15	15	18
T-I	0.3	9-M	15	17	24
		10-M	15	15	24
		11-M	15	16	39
		12-M	15	15	22
		13-F	15	19	27
		14-F	15	19	28
		15-F	16	16	21
		16-F	15	15	15
T-II	1.0	17-M	15	15	15
		18-M	15	15	20
		19-M	15	21	21
		20-M	15	14	17
		21-F	15	16	17
		22-F	15	14	15
		23-F	15	15	23
		24-F	15	15	18
T-III	3.0	25-M	15	15	15
		26-M	15	20	25
		27-M	15	20	28
		28-M	15	15	16
		29-F	15	15	23
		30-F	15	15	26
		31-F	15	15	16
		32-F	15	15	18

Note: fluorometric method by Levine and Hill.

G. Urine Analyses

The results of the urine analyses are presented in Tables XV through XVIII.

Urinalysis revealed no significant abnormalities at any of the levels tested.

TABLE XV

TEST MATERIAL: Levn-Lite

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Urine Analyses: Albumin, mg/100 ml

Group	Dietary Level (%)	Dog Number and Sex	Days: 0	42	84
UC	None	1-M	10	0	0
		2-M	10	0	0
		3-M	0	0	0
		4-M	10	10	0
		5-F	0	0	0
		6-F	10	11-50	0
		7-F	10	0	11-50
		8-F	0	0	0
T-I	0.3	9-M	0	10	0
		10-M	0	0	0
		11-M	10	0	0
		12-M	10	0	0
		13-F	0	11-50	0
		14-F	0	0	0
		15-F	10	10	0
		16-F	0	0	11-50
T-II	1.0	17-M	0	0	0
		18-M	0	10	0
		19-M	0	0	0
		20-M	0	0	0
		21-F	0	0	0
		22-F	0	0	0
		23-F	0	0	0
		24-F	0	0	0
T-III	3.0	25-M	0	0	0
		26-M	0	0	0
		27-M	0	0	11-50
		28-M	10	0	0
		29-F	0	0	0
		30-F	0	0	0
		31-F	10	0	0
		32-F	0	0	0

Note: BUMINTEST, Ames Company, Inc., Elkhart, Indiana.

TABLE XVI

TEST MATERIAL: Levn-Lite

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Urine Analyses: Glucose, mg/100 ml

Group	Dietary Level (%)	Dog Number and Sex	Days: 0	42	84
UC	None	1-M	0	0	0
		2-M	0	0	0
		3-M	0	0	0
		4-M	0	0	0
		5-F	0	0	0
		6-F	0	0	0
		7-F	0	0	0
		8-F	0	0	0
T-I	0.3	9-M	0	0	0
		10-M	0	0	0
		11-M	0	0	0
		12-M	0	0	0
		13-F	0	0	0
		14-F	0	0	0
		15-F	0	0	0
		16-F	0	0	0
T-II	1.0	17-M	0	0	0
		18-M	0	0	0
		19-M	0	0	0
		20-M	0	0	0
		21-F	0	0	0
		22-F	0	0	0
		23-F	0	0	0
		24-F	0	0	0
T-III	3.0	25-M	0	0	0
		26-M	0	0	0
		27-M	0	0	0
		28-M	0	0	0
		29-F	0	0	0
		30-F	0	0	0
		31-F	0	0	0
		32-F	0	0	0

Note: COMBISTIX, Ames Company, Inc., Elkhart, Indiana.

TABLE XVII

TEST MATERIAL: Levn-Lite

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Urine Analyses: pH

Group	Dietary Level (%)	Dog Number and Sex	Days: 0	42	84
UC	None	1-M	7	6	7
		2-M	8	7	6
		3-M	7	7	5
		4-M	7	6	8
		5-F	6	8	8
		6-F	8	7	7
		7-F	8	7	6
		8-F	6	8	7
T-I	0.3	9-M	6	8	7
		10-M	7	6	7
		11-M	6	8	5
		12-M	7	5	5
		13-F	6	7	8
		14-F	6	8	6
		15-F	6	9	6
		16-F	8	7	8
T-II	1.0	17-M	6	6	7
		18-M	6	6	5
		19-M	7	7	5
		20-M	6	6	7
		21-F	6	5	5
		22-F	6	6	5
		23-F	8	5	7
		24-F	7	8	7
T-III	3.0	25-M	6	5	5
		26-M	6	5	5
		27-M	6	5	6
		28-M	8	5	6
		29-F	6	5	7
		30-F	6	5	6
		31-F	6	5	6
		32-F	7	7	6

Note: COMBISTIX, Ames Company, Inc., Elkhart, Indiana.

TABLE XVIII

TEST MATERIAL: Levn-Lite

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Urine Analyses: Microscopic Elements,
average per high power field - Leukocytes

Group	Dietary Level (%)	Dog Number and Sex	Days: 0	42	84
UC	None	1-M	0	0	0
		2-M	0	0	0
		3-M	0	0	0
		4-M	0	0	0
		5-F	0	0	0
		6-F	0	0	0
		7-F	20	7	0
		8-F	3	0	0
T-I	0.3	9-M	0	0	0
		10-M	0	0	0
		11-M	0	0	0
		12-M	0	0	0
		13-F	5	15	0
		14-F	0	0	0
		15-F	0	0	0
		16-F	20	0	0
T-II	1.0	17-M	5	0	0
		18-M	0	0	0
		19-M	0	0	0
		20-M	0	0	0
		21-F	3	5	10
		22-F	0	3	0
		23-F	0	0	0
		24-F	0	0	0
T-III	3.0	25-M	0	0	0
		26-M	0	0	0
		27-M	0	0	0
		28-M	0	0	0
		29-F	10	0	5
		30-F	0	0	0
		31-F	0	0	0
		32-F	3	0	25

TABLE XVIII continued

TEST MATERIAL: Levn-Lite

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Urine Analyses: Microscopic Elements,
average per high power field - Erythrocytes

Group	Dietary Level (%)	Dog Number and Sex	Days: 0	42	84
UC	None	1-M	0	0	0
		2-M	0	0	0
		3-M	0	0	0
		4-M	0	0	0
		5-F	0	0	0
		6-F	0	0	0
		7-F	0	0	25*
		8-F	0	0	0
T-I	0.3	9-M	0	0	0
		10-M	0	0	0
		11-M	0	0	0
		12-M	0	0	0
		13-F	0	0	0
		14-F	0	0	0
		15-F	0	0	0
		16-F	0	0	0
T-II	1.0	17-M	0	0	0
		18-M	0	0	0
		19-M	0	0	0
		20-M	0	0	8
		21-F	0	0	0
		22-F	0	0	0
		23-F	0	0	0
		24-F	0	0	0
T-III	3.0	25-M	0	0	0
		26-M	0	0	0
		27-M	0	0	0
		28-M	5	0	0
		29-F	0	0	0
		30-F	0	0	0
		31-F	0	0	0
		32-F	0	0	0

* in estrus.

TABLE XVIII continued

TEST MATERIAL: Levn-Lite

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Urine Analyses: Microscopic Elements, average per high power field - Crystals

Group	Dietary Level (%)	Dog Number and Sex	Days: 0	42	84
UC	None	1-M	1-3	1-3	1-3
		2-M	1-3	1-3	1-3
		3-M	1-3	1-3	1-3
		4-M	1-3	1-3	1-3
		5-F	1-3	1-3	1-3
		6-F	1-3	1-3	1-3
		7-F	1-3	1-3	1-3
		8-F	1-3	1-3	1-3
T-I	0.3	9-M	1-3	1-3	1-3
		10-M	1-3	1-3	1-3
		11-M	1-3	1-3	1-3
		12-M	1-3	1-3	1-3
		13-F	1-3	1-3	1-3
		14-F	1-3	1-3	1-3
		15-F	1-3	1-3	1-3
		16-F	1-3	1-3	1-3
T-II	1.0	17-M	1-3	1-3	1-3
		18-M	1-3	1-3	1-3
		19-M	1-3	1-3	1-3
		20-M	1-3	1-3	1-3
		21-F	1-3	1-3	1-3
		22-F	1-3	1-3	1-3
		23-F	4	1-3	1-3
		24-F	4	1-3	1-3
T-III	3.0	25-M	1-3	1-3	1-3
		26-M	1-3	1-3	1-3
		27-M	1-3	1-3	1-3
		28-M	1-3	1-3	1-3
		29-F	1-3	1-3	1-3
		30-F	1-3	1-3	1-3
		31-F	1-3	1-3	1-3
		32-F	4	1-3	4

H. Pathologic Findings

1. Organ Weight Data

The organ weight data are presented in Tables XIX through XXVII.

No significant abnormalities were noted among any levels tested.

TABLE XIX

TEST MATERIAL: Levn-Lite

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Organ Weight Data: Liver

Group	Dietary Level (%)	Dog Number and Sex	Organ Weight (g)	Organ/Body Weight Ratio (g/1000 g)
UC	None	1-M	400.0	36.7
		2-M	402.1	34.7
		3-M	445.9	37.5
		4-M	364.4	36.8
		5-F	273.9	34.2
		6-F	301.7	33.9
		7-F	439.2	42.2
		8-F	294.4	37.7
T-I	0.3	9-M	394.2	32.0
		10-M	356.7	34.0
		11-M	319.8	34.8
		12-M	339.1	30.3
		13-F	268.6	38.4
		14-F	208.4	39.3
		15-F	360.3	31.3
		16-F	336.6	32.7
T-II	1.0	17-M	378.8	39.4
		18-M	399.2	32.4
		19-M	382.9	34.5
		20-M	338.8	30.2
		21-F	243.6	36.4
		22-F	222.1	25.2
		23-F	219.8	40.7
		24-F	326.9	34.4
T-III	3.0	25-M	277.9	33.9
		26-M	301.0	34.6
		27-M	417.1	33.6
		28-M	474.3	34.6
		29-F	205.4	39.5
		30-F	367.0	35.0
		31-F	269.4	33.2
		32-F	260.4	31.4

TABLE XX

TEST MATERIAL: Levn-Lite

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Organ Weight Data: Kidneys

Group	Dietary Level (%)	Dog Number and Sex	Organ Weight (g)	Organ/Body Weight Ratio (g/1000 g)
UC	None	1-M	68.7	6.30
		2-M	69.7	6.01
		3-M	68.4	5.75
		4-M	71.4	7.21
		5-F	48.5	6.06
		6-F	57.2	6.43
		7-F	64.8	6.23
		8-F	48.4	6.20
T-I	0.3	9-M	67.3	5.47
		10-M	57.5	5.48
		11-M	59.3	6.45
		12-M	57.0	5.09
		13-F	38.0	5.43
		14-F	31.0	5.85
		15-F	64.9	5.64
		16-F	55.2	5.36
T-II	1.0	17-M	64.6	6.73
		18-M	67.0	5.45
		19-M	78.5	7.07
		20-M	72.1	6.44
		21-F	44.5	6.64
		22-F	45.6	5.18
		23-F	35.2	6.52
		24-F	52.0	5.47
T-III	3.0	25-M	47.8	5.83
		26-M	63.0	7.24
		27-M	83.9	6.77
		28-M	89.5	6.53
		29-F	36.8	7.08
		30-F	75.1	7.15
		31-F	52.0	6.42
		32-F	50.3	6.06

TABLE XXI

TEST MATERIAL: Levn-Lite

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Organ Weight Data: Heart

Group	Dietary Level (%)	Dog Number and Sex	Organ Weight (g)	Organ/Body Weight Ratio (g/1000 g)
UC	None	1-M	91.9	8.43
		2-M	99.3	8.56
		3-M	107.6	9.04
		4-M	86.1	8.70
		5-F	74.3	9.29
		6-F	92.7	10.4
		7-F	78.3	7.53
		8-F	70.9	9.09
T-I	0.3	9-M	107.2	8.72
		10-M	92.3	8.79
		11-M	73.0	7.94
		12-M	97.3	8.69
		13-F	63.7	9.10
		14-F	47.7	9.00
		15-F	92.7	8.06
		16-F	98.8	9.59
T-II	1.0	17-M	77.4	8.06
		18-M	102.7	8.35
		19-M	98.2	8.85
		20-M	84.1	7.51
		21-F	76.8	11.5
		22-F	68.0	7.73
		23-F	43.5	8.06
		24-F	79.0	8.32
T-III	3.0	25-M	71.7	8.74
		26-M	79.1	9.09
		27-M	120.4	9.71
		28-M	100.7	7.35
		29-F	50.7	9.75
		30-F	98.7	9.40
		31-F	79.8	9.85
		32-F	79.2	9.54

TABLE XXII

TEST MATERIAL: Levn-Lite

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Organ Weight Data: Brain

Group	Dietary Level (%)	Dog Number and Sex	Organ Weight (g)	Organ/Body Weight Ratio (g/1000 g)
UC	None	1-M	76.7	7.04
		2-M	86.5	7.46
		3-M	90.5	7.60
		4-M	78.6	7.94
		5-F	82.0	10.2
		6-F	84.4	9.48
		7-F	78.0	7.50
		8-F	78.3	10.0
T-I	0.3	9-M	89.5	7.28
		10-M	82.9	7.90
		11-M	67.0	7.28
		12-M	80.5	7.19
		13-F	82.0	11.7
		14-F	65.0	12.3
		15-F	74.7	7.50
		16-F	87.8	8.52
T-II	1.0	17-M	77.7	8.09
		18-M	82.0	6.67
		19-M	86.4	7.78
		20-M	79.5	7.10
		21-F	83.0	12.4
		22-F	69.9	7.94
		23-F	71.8	13.3
		24-F	73.0	7.68
T-III	3.0	25-M	63.3	7.72
		26-M	76.8	8.83
		27-M	82.3	6.64
		28-M	83.5	6.10
		29-F	77.9	15.0
		30-F	81.5	7.76
		31-F	74.6	9.21
		32-F	84.4	10.2

TABLE XXIII

TEST MATERIAL: Levn-Lite

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Organ Weight Data: Spleen

Group	Dietary Level (%)	Dog Number and Sex	Organ Weight (g)	Organ/Body Weight Ratio (g/1000 g)
UC	None	1-M	22.2	2.04
		2-M	16.2	1.40
		3-M	35.8	3.01
		4-M	16.2	1.64
		5-F	16.9	2.11
		6-F	23.6	2.65
		7-F	25.4	2.44
		8-F	18.5	2.37
T-I	0.3	9-M	22.7	1.85
		10-M	19.8	1.89
		11-M	22.4	2.44
		12-M	20.0	1.79
		13-F	15.0	2.14
		14-F	10.3	1.94
		15-F	33.2	2.89
		16-F	23.8	2.31
T-II	1.0	17-M	17.3	1.80
		18-M	26.6	2.16
		19-M	34.2	3.08
		20-M	24.8	2.21
		21-F	16.1	2.40
		22-F	10.5	1.19
		23-F	10.2	1.89
		24-F	19.6	2.06
T-III	3.0	25-M	12.9	1.57
		26-M	18.0	2.07
		27-M	24.9	2.01
		28-M	20.3	1.48
		29-F	11.3	2.17
		30-F	25.1	2.39
		31-F	16.2	2.00
		32-F	19.8	2.39

TABLE XXIV

TEST MATERIAL: Levn-Lite

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Organ Weight Data: Gonads

Group	Dietary Level (%)	Dog Number and Sex	Organ Weight (g)	Organ/Body Weight Ratio (g/1000 g)
UC	None	1-M	5.4	0.495
		2-M	17.0	1.46
		3-M	15.5	1.30
		4-M	9.5	0.960
		5-F	0.586	0.0724
		6-F	0.621	0.0675
		7-F	0.509	0.0485
		8-F	0.489	0.0612
T-I	0.3	9-M	13.4	1.09
		10-M	6.9	0.657
		11-M	16.5	1.79
		12-M	10.5	0.938
		13-F	0.553	0.0758
		14-F	0.433	0.0759
		15-F	0.613	0.0524
		16-F	0.561	0.0529
T-II	1.0	17-M	6.6	0.688
		18-M	8.3	0.675
		19-M	6.2	0.559
		20-M	10.4	0.929
		21-F	0.400	0.0563
		22-F	0.505	0.0581
		23-F	0.346	0.0629
		24-F	0.536	0.0553
T-III	3.0	25-M	9.4	1.15
		26-M	12.5	1.44
		27-M	11.8	0.952
		28-M	20.7	1.51
		29-F	0.319	0.0540
		30-F	0.693	0.0608
		31-F	0.489	0.0550
		32-F	0.578	0.0672

TABLE XXV

TEST MATERIAL: Levn-Lite

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Organ Weight Data: Adrenal Glands

Group	Dietary Level (%)	Dog Number and Sex	Organ Weight (g)	Organ/Body Weight Ratio (g/1000 g)
UC	None	1-M	0.942	0.0856
		2-M	0.956	0.0803
		3-M	1.088	0.0884
		4-M	0.966	0.0947
		5-F	0.745	0.0919
		6-F	0.831	0.0903
		7-F	0.941	0.0896
		8-F	0.867	0.108
T-I	0.3	9-M	1.210	0.0953
		10-M	0.987	0.0905
		11-M	0.875	0.0931
		12-M	0.942	0.0805
		13-F	0.660	0.0904
		14-F	0.773	0.135
		15-F	1.148	0.0981
		16-F	1.003	0.0946
T-II	1.0	17-M	0.999	0.0999
		18-M	1.109	0.0894
		19-M	1.008	0.0876
		20-M	1.102	0.0967
		21-F	0.747	0.105
		22-F	0.863	0.0992
		23-F	0.609	0.111
		24-F	0.941	0.0970
T-III	3.0	25-M	0.932	0.112
		26-M	0.928	0.0987
		27-M	1.082	0.0865
		28-M	1.221	0.0866
		29-F	0.687	0.116
		30-F	1.108	0.0972
		31-F	0.866	0.0973
		32-F	0.805	0.0936

TABLE XXVI

TEST MATERIAL: Levn-Lite

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Organ Weight Data: Thyroid Gland

Group	Dietary Level (%)	Dog Number and Sex	Organ Weight (g)	Organ/Body Weight Ratio (g/1000 g)
UC	None	1-M	0.981	0.0892
		2-M	1.132	0.0951
		3-M	1.201	0.0976
		4-M	1.166	0.114
		5-F	0.844	0.104
		6-F	0.907	0.0986
		7-F	1.005	0.957
		8-F	0.804	0.100
T-I	0.3	9-M	1.146	0.0902
		10-M	1.000	0.0917
		11-M	0.926	0.0985
		12-M	1.146	0.0979
		13-F	0.701	0.0960
		14-F	0.510	0.0895
		15-F	1.108	0.0947
		16-F	1.100	0.104
T-II	1.0	17-M	0.982	0.0982
		18-M	1.107	0.0893
		19-M	1.086	0.0944
		20-M	1.086	0.0953
		21-F	0.752	0.106
		22-F	0.831	0.0955
		23-F	0.602	0.109
		24-F	0.913	0.0941
T-III	3.0	25-M	0.882	0.106
		26-M	0.821	0.0873
		27-M	1.206	0.0965
		28-M	1.301	0.0923
		29-F	0.618	0.105
		30-F	1.093	0.0959
		31-F	0.749	0.0842
		32-F	0.852	0.0991

TABLE XXVII

TEST MATERIAL: Levn-Lite

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Organ Weight Data: Pituitary Gland

Group	Dietary Level (%)	Dog Number and Sex	Organ Weight (g)	Organ/Body Weight Ratio (g/1000 g)
UC	None	1-M	0.083	0.00754
		2-M	0.098	0.00828
		3-M	0.085	0.00693
		4-M	0.072	0.00708
		5-F	0.064	0.00791
		6-F	0.077	0.00842
		7-F	0.073	0.00697
		8-F	0.052	0.00648
T-I	0.3	9-M	0.094	0.00743
		10-M	0.075	0.00692
		11-M	0.076	0.00805
		12-M	0.092	0.00783
		13-F	0.048	0.00652
		14-F	0.049	0.00853
		15-F	0.071	0.00752
		16-F	0.096	0.00904
T-II	1.0	17-M	0.064	0.00641
		18-M	1.090	0.00728
		19-M	0.094	0.00818
		20-M	0.083	0.00729
		21-F	0.051	0.00719
		22-F	0.060	0.00684
		23-F	0.045	0.00814
		24-F	0.071	0.00729
T-III	3.0	25-M	0.053	0.00641
		26-M	0.081	0.00867
		27-M	0.093	0.00743
		28-M	0.089	0.00789
		29-F	0.049	0.00838
		30-F	0.094	0.00823
		31-F	0.067	0.00752
		32-F	0.063	0.00735

2. Gross and Histologic Findings

The gross and histologic findings are presented in Tables XXVIII through XXXI. All tissues and organs not mentioned were normal.

The grading system used is as follows:

\+ = minimal or slight
++ = mild
+++ = moderate
++++ = severe

IBT No. J749
Monsanto

I have completed a histopathologic evaluation of tissues from 32 dogs of IBT No. J749. There are no changes that can be attributed to the test material or the test procedure. All of the findings noted are attributed to spontaneous disease.

Slides examined by: James F. Vondruska

James F. Vondruska, D.V.M.
Senior Group Leader
Pathology

Reviewed & approved by: D. E. Gordon

Donovan E. Gordon, D.V.M., Ph.D.
Diplomate, American College of
Veterinary Pathologists

TABLE XXVIII

TEST MATERIAL: Levn-Lite

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Gross and Histologic Findings

Untreated Control Group

Dog Number and Sex	Organ	Gross	Grade	Histologic	Grade
1-M	Liver	-	-	Focal lymphoid infiltration	+
	Lungs	-	-	Focal interstitial pneumonia	++
	Prostate	-	-	Chronic focal prostatitis	++
	Spleen	-	-	Hemosiderosis	+
2-M	Lungs	-	-	Chronic interstitial pneumonia	++
3-M	Heart	-	-	Congestion	+
	Liver	-	-	Focal lymphoid infiltration	+
	Lungs	-	-	Focal interstitial pneumonia	++
4-M	Liver	-	-	Congestion	+
	Lungs	-	-	Chronic interstitial pneumonia	++
	Spleen	-	-	Hemosiderosis	+

TABLE XXVIII continued

TEST MATERIAL: Levn-Lite

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Gross and Histologic Findings

Untreated Control Group

Dog Number and Sex	Organ	Gross	Grade	Histologic	Grade
5-F	Liver	-	-	Congestion	++
				Focal lymphoid infiltration	+
	Lungs	-	-	Chronic interstitial pneumonia	+
				Hyperemia	+
6-F	Liver	-	-	Congestion	+
	Lungs	-	-	Chronic interstitial pneumonia	+
	Uterus	-	-	In estrus	-
7-F	Ovaries	-	-	Proestrus	-
	Liver	-	-	Congestion	++
8-F	Lungs	-	-	Chronic interstitial pneumonia	+

TABLE XXIX

TEST MATERIAL: Levn-Lite

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Gross and Histologic Findings

Test Group I: 0.3 percent

Dog Number and Sex	Organ	Gross	Grade	Histologic	Grade
9-M	-	-	-	-	-
10-M	Lungs	-	-	Chronic interstitial pneumonia	+
11-M	Liver	-	-	Congestion	+
	Lungs	-	-	Chronic interstitial pnuemonia	+
12-M	Liver	-	-	Congestion	+
	Lungs	-	-	Focal interstitial pneumonia	+
13-F	Adrenal glands	-	-	Congestion	+
	Liver	-	-	Congestion	+
	Lungs	-	-	Chronic interstitial pneumonia	+
14-F	Liver	-	-	Congestion	+
	Lungs	-	-	Focal interstitial pneumonia	+
	Trachea	-	-	Focal lymphoid infiltration	+

TABLE XXIX continued

TEST MATERIAL: Levn-Lite

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Gross and Histologic Findings

Test Group I: 0.3 percent

Dog Number and Sex	Organ	Gross	Grade	Histologic	Grade
15-F	Lungs	-	-	Chronic interstitial pneumonia	+
16-F	Lungs	-	-	Chronic interstitial pneumonia	++

TABLE XXX

TEST MATERIAL: Levn-Lite

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Gross and Histologic Findings

Test Group II: 1.0 percent

Dog Number and Sex	Organ	Gross	Grade	Histologic	Grade
17-M	Lungs	-	-	Congestion	+
18-M	Liver	-	-	Focal lymphoid infiltration	+
	Lungs	-	-	Chronic interstitial pneumonia	+
	Prostate	-	-	Chronic focal prostatitis	+
19-M	Liver	-	-	Congestion	+
20-M	-	-	-	-	-
21-F	Lungs	-	-	Chronic interstitial pneumonia	+
22-F	Lungs	Consolidation	+	Chronic interstitial pneumonia	+
23-F	Liver	-	-	Focal lymphoid infiltration	++
	Lungs	-	-	Chronic interstitial pneumonia	+++
24-F	Kidneys	-	-	Congestion	+
	Liver	-	-	Congestion	+
				Focal lymphoid infiltration	+
	Lungs	-	-	Chronic interstitial pneumonia	++

TABLE XXXI

TEST MATERIAL: Levn-Lite

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Gross and Histologic Findings

Test Group III: 3.0 percent

Dog Number and Sex	Organ	Gross	Grade	Histologic	Grade
25-M	Liver	-	-	Focal lymphoid infiltration	+
	Lungs	-	-	Chronic interstitial pneumonia	+
26-M	Liver	-	-	Congestion	++
	Prostate	-	-	Chronic focal prostatitis	++
27-M	Lungs	-	-	Chronic interstitial pneumonia	+
28-M	-	-	-	-	-
29-F	Adrenal glands	-	-	Hyperemia	+
	Liver	-	-	Focal lymphoid infiltration	+
	Lungs	-	-	Hyperemia	++
30-F	Lungs	-	-	Hyperemia	+
				Chronic interstitial pneumonia	+

TABLE XXXI continued

TEST MATERIAL: Levn-Lite

90-Day Subacute Oral Toxicity Study - Beagle Dogs

Gross and Histologic Findings

Test Group III: 3.0 percent

Dog Number and Sex	Organ	Gross	Grade	Histologic	Grade
31-F	Liver	-	-	Congestion	+
	Lungs	-	-	Focal bronchopneumonia	++
				Chronic interstitial pneumonia	+
32-F	Lungs	-	-	Chronic interstitial pneumonia	+

Industrial BIO-TEST Laboratories, Inc.
1810 FRONTAGE ROAD
NORTHBROOK, ILLINOIS 60062

REPORT TO

MONSANTO COMPANY

90-DAY SUBACUTE ORAL TOXICITY STUDY WITH LEVN-LITE IN ALBINO RATS

BTL-71-49

JUNE 28, 1972

IBT NO. B747

Industrial **BIO-TEST** *Laboratories, Inc.*
1810 FRONTAGE ROAD
NORTHBROOK, ILLINOIS 60062

TOXICOLOGY
ENVIRONMENTAL SCIENCES
CHEMISTRY
PLANT SCIENCES
MEDICAL SCIENCES

AREA CODE 312
TELEPHONE 272-3030

June 28, 1972

Dr. George J. Levinskas
Monsanto Company
800 N. Lindbergh Boulevard
St. Louis, Missouri 63166

Dear Dr. Levinskas:

Re: IBT No. B747 - 90-Day Subacute Oral Toxicity Study with Levn-Lite in Albino Rats

We are submitting herewith our laboratory report dated June 28, 1972, prepared in connection with the above study.

Very truly yours,

J. C. Calandra
President

JCC/slg

REPORT TO

MONSANTO COMPANY

90-DAY SUBACUTE ORAL TOXICITY STUDY WITH LEVN-LITE IN ALBINO RATS

BTL-71-49

JUNE 28, 1972

IBT NO. B747

I. Introduction

A sample identified as Levn-Lite was received from the Monsanto Company for the purpose of conducting a 90-day subacute oral toxicity study using albino rats as test animals. The following report presents the results of this investigation.

II. Summary

A 90-day subacute oral toxicity study was conducted with groups of albino rats fed Levn-Lite at dietary levels of 0.3, 1.0 and 3.0 percent. Results obtained from microscopic examination of tissues and organs disclosed microconcretions in the renal tubules of the female rats from all three test groups. These concretions are believed to be related to the test material since they are absent in the control animals and since the incidence and severity of this finding appear to be dose related. No abnormalities were observed in any of the following parameters:

Body Weight Gains
Food Consumption
Hematologic Studies
Clinical Blood Chemistry Studies
Urine Analyses
Gross Pathologic Studies
Organ Weights and Ratios

Respectfully submitted,

INDUSTRIAL BIO-TEST LABORATORIES, INC.

Report prepared by:

Philip S. Smith

Philip S. Smith, B.S.
Assistant Toxicologist
Rat Toxicity

Report approved by:

James B. Plank

James B. Plank
Senior Group Leader
Rat Toxicity

Paul L. Wright

Paul L. Wright, Ph.D.
Section Head, Toxicology

M. L. Keplinger

M. L. Keplinger, Ph.D.
Manager, Toxicology

slg

III. Procedure

A. Experimental Animals

The animals employed in the study were Charles River strain* albino rats. One hundred and twenty rats (60 males and 60 females) were selected for the experiment and housed individually in standard, wire-bottomed steel rat cages. Each cage bore a color-coded card identifying the animal with respect to project number, dietary level assignment, individual animal number and sex.

B. Organization of Groups

A structural outline of the experiment is shown in Table I.

TABLE I

TEST MATERIAL: Levn-Lite

90-Day Subacute Oral Toxicity Study - Albino Rats

Outline of Experiment

Group	Number of Animals Males	Females	Dietary Level (percent)
Control	15	15	None Administered
T-I	15	15	0.3
T-II	15	15	1.0
T-III	15	15	3.0

* Charles River Breeding Laboratories, Inc., North Wilmington, Mass.

C. Body Weights

Each animal used in the study was weighed on the first day of the test and at weekly intervals thereafter. The weights were recorded and served as an index to growth. Weight gains were computed at the conclusion of the 90-day test period and the data subjected to statistical analyses.

D. Food Consumption and Diet Preparation

Food consumption data were collected individually for five rats of each sex in every group weekly during the study and the data recorded.

The diet for any given group was prepared by blending the appropriate amount of Levn-Lite with standard rat ration in a Hobart Mixer.

Fresh diets were prepared each week. Each rat was offered an amount of diet sufficient for one weeks' ad libitum feeding. However, checks were made periodically to ensure that the food jars were not empty.

E. Mortality and Reactions

Abnormal reactions and deaths were recorded daily during the investigation.

F. Hematologic, Clinical Blood Chemistry Studies and Urine Analyses

Blood and urine samples collected individually from ten rats of each sex from both the control and T-III groups after 45 and 84 days of feeding were analyzed for the following:

1. Hematologic Studies

   a. Hematocrit Value
   b. Erythrocyte Count
   c. Hemoglobin Concentration
   d. Total Leukocyte Count
   e. Differential Leukocyte Count

2. Clinical Blood Chemistry Studies

   a. Blood Urea Nitrogen (BUN) Concentration
   b. Serum Alkaline Phosphatase (SAP) Activity
   c. Serum Glutamic-Pyruvic Transaminase (SGPT) Activity
   d. Fasted Blood Glucose Concentration

3. Urine Analyses

   a. Glucose Concentration
   b. Albumin Concentration
   c. Microscopic Elements Examination
   d. pH
   e. Specific Gravity

G. Pathologic Studies

Following 90 days of feeding, all surviving rats were sacrificed by carbon dioxide asphyxiation and autopsied. Animals which died during the study were examined grossly unless examination was precluded by postmortem autolysis. At the time of gross examination a complete set of organs and other tissues was removed from each rat and preserved in formalin solution. Also at autopsy the weights of the liver, kidneys, spleen, gonads, heart and brain of each rat were determined and recorded.

Microscopic examination of tissues taken from ten rats of each sex from both the control and T-III groups was conducted. The following tissues, stained with Hematoxylin-Eosin, were included: esophagus,

stomach (cardia, fundus and pylorus), small intestine (duodenum, jejunum and ileum), cecum, colon, liver, kidneys, spleen, pancreas, urinary bladder, pituitary gland, adrenal gland, testes, seminal vesicle, ovary, bone marrow, thyroid gland, parathyroid gland, salivary gland, prostate gland, heart, aorta, lung, lymph node (cervical and mesenteric), skeletal muscle, peripheral nerve, bone (femur), spinal cord, uterus, trachea, eye, optic nerve and brain (cerebrum, cerebellum and pons).

H. Organ Weights, Organ to Body Weight and Organ to Brain Weight Ratios

Statistical analyses were conducted upon the absolute organ weights and their corresponding ratios to the weight of the body and brain. An Analysis of Variance was conducted first and any significant effects disclosed by that treatment were further studied by "t"-tests.

IV. Results

A. Body Weights

Body weight data collected during the 90-day test period are summarized in Table II. Also included in the table are 90-day average total weight gains.

Statistical comparisons of final body weights and total weight gains revealed no significant differences between test and control rats.

TABLE II

TEST MATERIAL: Levn-Lite

90-Day Subacute Oral Toxicity Study - Albino Rats

Body Weight and Total Weight Gain Data

Summary of Mean Values

Dietary Level (percent)	Sex	Body Weight (grams) Week: 0	1	2	3	4	5	6	7	8	9	10	11	12	13	Total Weight Gain (grams/rat)
Control	M	185	242	298	349	361	391	417	437	458	481	515	518	521	522	337
	F	150	185	211	232	245	253	260	275	281	291	304	301	318	331	181
0.3	M	185	241	297	345	377	414	439	464	475	519	532	539	555	580	395
	F	150	191	215	232	243	254	267	273	280	292	298	297	306	312	162
1.0	M	186	247	309	343	371	402	425	447	456	481	490	505	521	541	355
	F	149	183	208	229	240	248	263	271	272	279	285	295	301	306	157
3.0	M	187	242	303	352	382	407	425	449	468	499	507	516	527	542	355
	F	150	182	210	225	233	247	262	264	273	290	290	286	290	298	148

B. Food Consumption

Food consumption data collected during the 90-day test period are summarized in Table III.

Test rats ate amounts of food comparable to that consumed by control rats.

TABLE III

TEST MATERIAL: Levn-Lite

90-Day Subacute Oral Toxicity Study - Albino Rats

Food Consumption Data

Summary of Mean Values

Dietary Level (percent)	Sex	Food Consumption (grams/rat/seven days) Week: 1	2	3	4	5	6	7	8	9	10	11	12	13	Total Food Consumption (grams/rat)
Control	M	154	148	169	163	191	204	124	206	185	201	186	191	174	2296
	F	108	117	125	117	147	149	97	148	160	128	117	127	165	1705
0.3	M	151	102	160	224	153	202	168	214	192	194	182	187	189	2318
	F	113	111	98	163	92	132	108	131	120	123	103	123	149	1566
1.0	M	155	139	130	155	181	182	159	191	175	176	176	119	189	2127
	F	128	117	112	109	141	141	82	138	122	123	75	125	157	1570
3.0	M	167	137	174	159	159	202	137	198	183	184	131	178	185	2194
	F	122	106	117	115	119	129	114	167	133	123	142	135	158	1680

C. Mortaltiy and Reactions

Three deaths occurred during the study. All of these deaths resulted from trauma incurred during the collection of blood samples.

No untoward behavioral reactions were noted among any of the animals employed in the study.

D. Hematologic Studies

The results of the hematologic studies conducted on blood samples taken from ten rats of each sex in the control and T-III groups after 45 and 84 days of feeding are summarized in Tables IV and V.

No outstanding differences between test and control rats were noted with respect to any of the parameters investigated.

TABLE IV

TEST MATERIAL: Levn-Lite

90-Day Subacute Oral Toxicity Study - Albino Rats

Hematologic Data

Summary of Mean Values

Dietary Level (percent)	Sex	Total Leukocyte Count (thousands/mm^3) Day: 45	84	Erythrocyte Count (millions/mm^3) Day: 45	84	Hemoglobin Concentration (g/100 ml) Day: 45	84	Hematocrit Value (%) Day: 45	84
Control	M	17.1	15.9	7.72	8.08	15.7	15.9	39.3	40.5
	F	14.7	9.9	7.70	7.52	15.5	15.7	38.4	37.9
3.0	M	15.0	14.2	7.75	8.23	15.9	16.2	39.4	41.0
	F	14.6	9.2	7.78	7.77	15.9	15.7	39.1	39.3

TABLE V

TEST MATERIAL: Levn-Lite

90-Day Subacute Oral Toxicity Study - Albino Rats

Hematologic Data

Summary of Mean Values

Dietary Level (percent)	Sex	Differential Leukocyte Count (Number of Cells per Hundred)									
		Lymphocytes Day:		Neutrophils Day:		Monocytes Day:		Eosinophils Day:		Basophils Day:	
		45	84	45	84	45	84	45	84	45	84
Control	M	90.6	86.7	8.6	11.1	0.8	1.7	0.0	0.5	0.0	0.0
	F	83.2	85.5	15.2	12.6	0.8	1.0	0.8	0.9	0.0	0.0
3.0	M	84.4	83.1	13.2	13.8	1.6	2.1	0.8	1.0	0.0	0.0
	F	85.6	87.1	12.2	11.7	1.0	1.0	1.2	0.2	0.0	0.0

E. Clinical Blood Chemistry Studies

The results of the clinical chemistry studies conducted on blood samples obtained from ten rats of each sex in the control and T-III groups after 45 and 84 days of feeding are summarized in Tables VI and VII.

Values for test rats were not different from those of control rats.

TABLE VI

TEST MATERIAL: Levn-Lite

90-Day Subacute Oral Toxicity Study - Albino Rats

Clinical Blood Chemistry Data

Summary of Mean Values

Dietary Level (percent)	Sex	Serum Alkaline Phosphatase Activity (King-Armstrong Units) Day: 45	Day: 84	Serum Glutamic-Pyruvic Transaminase Activity (Dade Units) Day: 45	Day: 84
Control	M	31	24	30	27
	F	20	12	24	28
3.0	M	33	23	26	32
	F	18	12	26	21

TABLE VII

TEST MATERIAL: Levn-Lite

90-Day Subacute Oral Toxicity Study - Albino Rats

Clinical Blood Chemistry Data

Summary of Mean Values

Dietary Level (percent)	Sex	Blood Urea Nitrogen Concentration (mgs %) Day: 45	84	Fasted Blood Glucose Concentration (mgs %) Day: 45	84
Control	M	16	15	129	141
	F	17	14	130	137
3.0	M	16	14	144	153
	F	16	14	154	143

F. Urine Analyses

The results of the periodic examinations of urine specimens collected from ten rats of each sex in the control and T-III groups after 45 and 84 days of feeding are summarized in Table VIII.

No significant differences between the urine of the test rats and control rats were observed.

TABLE VIII

TEST MATERIAL: Levn-Lite

90-Day Subacute Oral Toxicity Study - Albino Rats

Urine Analysis Data

Summary of Mean Values

Dietary Level (percent)	Sex	Glucose Day: 45	Glucose Day: 84	Albumin Day: 45	Albumin Day: 84	Microscopic Elements Day: 45	Microscopic Elements Day: 84	pH Day: 45	pH Day: 84	Specific Gravity Day: 45	Specific Gravity Day: 84
Control	M	n	n	t	n	+1	+2	7.0	6.8	1.025	1.039
	F	n	n	n	n	+2	+1	7.8	6.8	1.027	1.036
3.0	M	n	n	n	t	+1	t	6.8	6.8	1.021	1.053
	F	n	n	n	t	+1	+1	6.6	6.6	1.025	1.032

Glucose and Albumin

n = negative
t = trace; less than 30 mg/100 ml urine
+1 = 30 to 100 mg/100 ml urine
+2 = 100 to 300 mg/100 ml urine
+3 = 300 to 500 mg/100 ml urine
+4 = more than 500 mg/100 ml urine

Microscopic Elements

N = normal
t = minimal or trace amounts
+1 = slight amounts
+2 = moderate amounts
+3 = large amounts
+4 = extreme amounts

G. Pathologic Studies

1. Gross Pathologic Findings

No outstanding differences were noted between test and control rats upon gross pathological examination.

2. Organ Weight and Organ to Body and Organ to Brain Weight Ratio Data

The results of the statistical analyses conducted on absolute organ weights, organ to body weight and organ to brain weight ratios are summarized in Tables IX through XIV.

Significant differences between a test group and the control group are designated by asterisks following the test values.

The number of statistically significant intergroup differences which were noted was considered to be normal for a random population of albino rats. The lack of any consistent dose or sex related response and the absence of any deleterious histopathologic changes further substantiate that none of the intergroup differences were related to the ingestion of Levn-Lite.

TABLE IX

TEST MATERIAL - LEVN-LITE

90-DAY SUBACUTE ORAL TOXICITY STUDY

ALBINO RATS

ORGAN WEIGHT AND RATIO DATA

SUMMARY OF MEAN VALUES

ORGAN - LIVER

DIETARY LEVEL (PER CENT)	ORGAN WEIGHT (GM)		ORGAN/BODY WEIGHT RATIO (GM/100 GM)		ORGAN/BRAIN WEIGHT RATIO (GM/GM)	
	MALES	FEMALES	MALES	FEMALES	MALES	FEMALES
NONE	18.140	10.064	3.4790	3.2526	8.8872	5.0896
0.3	19.513	10.862	3.3432	3.3496	9.4149	5.7542
1.0	19.024	9.034	3.4991	2.9358	9.2539	4.8977
3.0	17.015	10.119	3.1883	3.3999	8.1248	5.2737

O STATISTICALLY SIGNIFICANT TREATMENT EFFECTS FOUND.

TABLE X

TEST MATERIAL - LEVN-LITE

90-DAY SUBACUTE ORAL TOXICITY STUDY

ALBINO RATS

ORGAN WEIGHT AND RATIO DATA

SUMMARY OF MEAN VALUES

ORGAN - KIDNEYS

DIETARY LEVEL (PER CENT)	ORGAN WEIGHT (GM)		ORGAN/BODY WEIGHT RATIO (GM/100 GM)		ORGAN/BRAIN WEIGHT RATIO (GM/GM)	
	MALES	FEMALES	MALES	FEMALES	MALES	FEMALES
NONE	3.666	2.117	0.7019	0.6798	1.8015	1.0695
0.3	3.713	2.134	0.6364	0.6686	1.7939	1.1141
1.0	3.739	2.067	0.693C	0.6688	1.8148	1.1259
3.0	3.644	2.227	0.6812	0.7468*	1.7462	1.1639

* STATISTICALLY SIGNIFICANT DIFFERENCE AT THE 95 PERCENT CONFIDENCE LEVEL.

TABLE XI

TEST MATERIAL - LEVN-LITE

90-DAY SUBACUTE ORAL TOXICITY STUDY

ALBINC RATS

ORGAN WEIGHT ANC RATIO CATA

SUMMARY CF MEAN VALUES

CRGAN - SPLEEN

DIETARY LEVeL (PER CENT)	CRGAN WEIGHT (GM)		CRGAN/BCDY WEIGHT RATIC (GM/100 GM)		CRGAN/BRAIN WEIGHT RATIC (GM/GM)	
	MALES	FEMALES	MALES	FEMALES	MALES	FEMALES
NONE	0.936	0.618	0.1805	0.2031	C.4599	C.3087
C.3	C.973	0.599	0.1681	0.1869	C.4684	C.3119
1.C	C.884	0.482	0.1641	0.1588	C.4315	C.2609
3.0	C.926	0.578	0.1737	0.1934	C.4445	C.3025

NC STATISTICALLY SIGNIFICANT TREATMENT EFFECTS FCUNC.

TABLE XII

TEST MATERIAL - LEVN-LITE

90-DAY SUBACUTE ORAL TOXICITY STUDY

ALBINO RATS

ORGAN WEIGHT AND RATIO DATA

SUMMARY OF MEAN VALUES

ORGAN - GONADS

DIETARY LEVEL (PER CENT)	ORGAN WEIGHT (GM)		ORGAN/BODY WEIGHT RATIO (GM/100 GM)		ORGAN/BRAIN WEIGHT RATIO (GM/GM)	
	MALES	FEMALES	MALES	FEMALES	MALES	FEMALES
NONE	3.611	0.077	0.6924	0.0253	1.7724	0.0392
0.3	3.537	0.066	0.6064**	0.0208	1.7115	0.0348
1.0	3.566	0.083	0.6653	0.0271	1.7317	0.0449
3.0	3.638	0.085	0.6823	0.0285	1.7452	0.0449

** STATISTICALLY SIGNIFICANT DIFFERENCE AT THE 99 PERCENT CONFIDENCE LEVEL.

TABLE XIII

TEST MATERIAL - LEVN-LITE

90-DAY SUBACUTE ORAL TOXICITY STUDY

ALBINC RATS

ORGAN WEIGHT ANC RATIO DATA

SUMMARY CF MEAN VALUES

ORGAN - HEART

DIETARY LEVEL (PER CENT)	ORGAN WEIGHT (GM)		ORGAN/BCDY WEIGHT RATIC (GM/100 GM)		ORGAN/BRAIN WEIGHT RATIC (GM/GM)	
	MALES	FEMALES	MALES	FEMALES	MALES	FEMALES
NCNE	1.613	1.078	0.3095	0.3484	C.7937	C.5447
C.3	1.696	1.093	0.2904	0.3417	C.8199	C.5724
1.0	1.674	1.025	0.3096	0.3365	C.8163	C.5574
3.0	1.6C8	1.C01	0.3015	0.3367	C.7707	C.523C

STATISTICALLY SIGNIFICANT TREATMENT EFFECTS FCUNC.

TABLE XIV

TEST MATERIAL - LEVN-LITE

90-DAY SUBACUTE ORAL TOXICITY STUDY

ALBINC RATS

ORGAN WEIGHT ANC RATIO CATA

SUMMARY CF MEAN VALUES

CRGAN - BRAIN

DIETARY LEVEL (PER CENT)	CRGAN WEIGHT (GM) MALES	FEMALES	CRGAN/BCDY WEIGHT RATIO (GM/100 GM) MALES	FEMALES	CRGAN/BRAIN WEIGHT RATIC (GM/GM) MALES	FEMALES
NCNE	2.C41	1.982	0.3914	C.6431	1.COCO	1.CCCC
0.3	2.C76	1.917	0.3575*	0.6038	1.COCO	1.CCCC
1.0	2.C63	1.838**	0.3841	C.6098	1.0000	1.CCCO
3.0	2.C86	1.919	0.3914	C.6457	1.OOCC	1.CCCC

* STATISTICALLY SIGNIFICANT DIFFERENCE AT THE 95 PERCENT CONFIDENCE LEVEL.
** STATISTICALLY SIGNIFICANT DIFFERENCE AT THE 99 PERCENT CONFIDENCE LEVEL.

3. Histopathologic Findings

Histopathologic examination of tissues and organs taken from ten rats of each sex in both the control and T-III groups was conducted. Microscopic examination of sections of kidneys taken from ten rats of each sex from the T-II group and from all female rats from the T-I group was also conducted.

Tables XV through XVIII list all histopathologic changes noted.

IBT No. B747
Monsanto Company

I have completed a histopathologic evaluation of a series of rat tissues from IBT No. B747. There are microconcretions present in the renal tubules of the female rats from all three dose levels. These concretions are located in the tubules at the corticomedullary junction and they consist of an amorphous material which shattered on sectioning. They are blue in color and are probably calcified. These concretions are believed to be related to the test material since they are absent in the control animals and since the incidence and severity of this finding appear to be dose related.

The other lesions observed are those of spontaneous disease and they are not unusual for the albino rat.

Ward R Richter

Ward R. Richter, D.V.M., M.S.
Diplomate, American College of
Veterinary Pathologists

TABLE XV

TEST MATERIAL: Levn-Lite

90-Day Subacute Oral Toxicity Study - Albino Rats

Histopathologic Changes

Group: Control

Number of Animals	Organ Examined	Findings	Incidence	Average Grade
10 Males	Trachea	Chronic tracheitis	6	1.0
	Lung	Chronic murine pneumonia	3	1.0
	Colon	Parasites	1	1.0
	Kidney	Focal lymphoid infiltration	3	1.0
	Urinary bladder	Mucoid plug	6	1.0
10 Females	Lung	Chronic murine pneumonia	1	1.0
	Colon	Parasites	1	1.0

All other tissues and organs, as listed on pages 5 and 6, were normal histologically.

Grading System

0.5 = minimal
1.0 = slight
2.0 = mild
3.0 = moderate
4.0 = severe
5.0 = extreme

TABLE XVI

TEST MATERIAL: Levn-Lite

90-Day Subacute Oral Toxicity Study - Albino Rats

Histopathologic Changes

Group: T-I (0.3%)

Number of Animals	Organ Examined	Findings	Incidence	Average Grade
15 Females	Kidney	Microconcretions	4	0.5
		Hydronephrosis	1	2.0
		Focal lymphoid infiltration	1	2.0

Grading System

0.5 = minimal
1.0 = slight
2.0 = mild
3.0 = moderate
4.0 = severe
5.0 = extreme

TABLE XVII

TEST MATERIAL: Levn-Lite

90-Day Subacute Oral Toxicity Study - Albino Rats

Histopathologic Changes

Group: T-II (1.0%)

Number of Animals	Organ Examined	Findings	Incidence	Average Grade
10 Males	Kidney	No findings	-	-
10 Females	Kidney	Microconcretions	5	2.0
		Focal lymphoid infiltration	1	2.0

Grading System

0.5 = minimal
1.0 = slight
2.0 = mild
3.0 = moderate
4.0 = severe
5.0 = extreme

TABLE XVIII

TEST MATERIAL: Levn-Lite

90-Day Subacute Oral Toxicity Study - Albino Rats

Histopathologic Changes

Group: T-III (3.0%)

Number of Animals	Organ Examined	Findings	Incidence	Average Grade
10 Males	Trachea	Chronic tracheitis	4	1.0
	Lung	Chronic murine pneumonia	5	1.0
	Kidney	Focal lymphoid infiltration	1	1.0
	Urinary bladder	Mucoid plug	1	1.0
10 Females	Trachea	Chronic tracheitis	4	1.0
	Colon	Parasites	1	1.0
	Kidney	Microconcretions	9	1.0
		Focal lymphoid infiltration	1	1.0

All other tissues and organs, as listed on pages 5 and 6, were normal histologically.

Grading System

0.5 = minimal
1.0 = slight
2.0 = mild
3.0 = moderate
4.0 = severe
5.0 = extreme

PROJECT NO. BTL-71-49
REPORT FILE

Replacement
original

Industrial **BIO-TEST** *Laboratories, Inc.*
1810 FRONTAGE ROAD
NORTHBROOK, ILLINOIS 60062

3

REPORT TO

MONSANTO COMPANY

90-DAY SUBACUTE ORAL TOXICITY STUDY WITH LEVN-LITE IN FEMALE ALBINO RATS

BTL-71-49

MAY 11, 1973

IBT NO. B2423

Industrial BIO-TEST Laboratories, Inc.
1810 FRONTAGE ROAD
NORTHBROOK, ILLINOIS 60062

February 1, 1974

Dr. Paul L. Wright
Manager, Toxicology
Medical Department
Monsanto Company
800 North Lindbergh Boulevard
St. Louis, Missouri 63166

Dear Dr. Wright:

Re: IBT No. B2423 - 90-Day Subacute Oral Toxicity Study with Levn-Lite in Female Albino Rats - BTL-71-49

We are enclosing herewith our revised laboratory report dated May 11, 1973, prepared in connection with the above study.

Very truly yours,

J. C. Calandra

J. C. Calandra
President

JCC:bp

REPORT TO

MONSANTO COMPANY

90-DAY SUBACUTE ORAL TOXICITY STUDY WITH LEVN-LITE IN FEMALE ALBINO RATS

BTL-71-49

MAY 11, 1973

IBT NO. B2423

I. Introduction

A sample identified as Levn-Lite was received from the Monsanto Company for the purpose of conducting a 90-day subacute oral toxicity study using albino rats as test animals. The following report presents the results of this investigation.

II. Summary

A 90-day subacute oral toxicity study was conducted with groups of female albino rats fed Levn-Lite at dietary levels of 300 and 1,000 ppm. No abnormalities were observed in survival, growth rate or kidney weights in either of the test groups.

Respectfully submitted,

INDUSTRIAL BIO-TEST LABORATORIES, INC.

Report prepared by: M. S. Reyna

M. S. Reyna, B.S.
Group Leader
Rat Toxicity

Report approved by: Gerald L Kennedy Jr

Gerald L. Kennedy, Jr., B.S.
Section Head, Toxicology

M. L. Keplinger

M. L. Keplinger, Ph.D.
Manager, Toxicology

msh:lam

III. Procedure

A. Experimental Animals

The animals employed in the study were Charles River strain* albino rats. Forty-five female rats were selected for the experiment and housed individually in standard, wire-bottomed, steel rat cages. Each cage bore a color-coded card identifying the animal with respect to project number, dietary level assignment, and individual animal number.

B. Organization of Groups

A structural outline of the experiment is shown in Table I.

TABLE I

TEST MATERIAL: Levn-Lite

90-Day Subacute Oral Toxicity Study - Female Albino Rats

Outline of Experiment

Group	Number of Animals	Dietary Level (ppm)
Control	15	None
T-I	15	300
T-II	15	1,000

* Charles River Breeding Laboratories, Inc., North Wilmington, Mass.

C. Body Weights

Each animal used in the study was weighed on the first day of the test and at monthly intervals thereafter. The weights were recorded and served as an index to growth. Weight gains were computed at the conclusion of the 90-day test period.

D. Diet Preparation

The diet for any given group was prepared by blending the appropriate amount of Levn-Lite with standard rat ration in a Hobart Mixer.

Fresh diets were prepared each week. Each rat was offered an amount of diet sufficient for one week's ad libitum feeding. However, checks were made periodically to ensure that the food jars were not empty.

E. Mortality and Reactions

Abnormal reactions and deaths were recorded daily during the investigation.

F. Pathologic Studies

Following 90 days of feeding, all surviving rats were sacrificed by carbon dioxide asphyxiation and autopsied. Animals which died during the study were examined grossly unless examination was precluded by postmortem autolysis. At the time of the final sacrifice the kidneys from each rat were removed and preserved in formalin solution. Also at autopsy the weights of the kidneys were determined and recorded.

G. Kidney Weights and Kidney to Body Weight Ratios

Statistical analyses were conducted upon the absolute kidney weights and their corresponding ratios to the weight of the body. An Analysis of Variance was conducted first and any significant effects disclosed by that treatment were further studied by Student's "t"-tests.

IV. Results

A. Body Weights

Body weight data collected during the 90-day test period are summarized in Table II. Also included in the table are 90-day average total weight gains.

Comparisons of final body weights and total weight gains revealed no significant differences between test and control rats.

TABLE II

TEST MATERIAL: Levn-Lite

90-Day Subacute Oral Toxicity Study - Female Albino Rats

Body Weight and Total Weight Gain Data

Summary of Mean Values

Dietary Level (ppm)	Body Weight (g) Month: 0	1	2	3	Total Weight Gain (g/rat)
Control	122	231	285	302	180
300	122	234	292	311	189
1,000	122	231	281	301	179

B. Mortality and Reactions

Five deaths occurred during the study. All of these deaths resulted from natural causes.

No untoward behavioral reactions were noted among any of the animals employed in the study.

C. Pathologic Studies

1. Gross Pathologic Findings

No outstanding differences were noted between test and control rats upon gross pathological examination.

2. Kidney Weight and Kidney to Body Weight Ratio Data

The results of the statistical analyses conducted on absolute kidney weights and kidney to body weight ratios are summarized in Table III. The individual kidney weights are listed in Table IV.

There were no statistically significant intergroup differences.

TABLE III

TEST MATERIAL: LEVN-LITE

90-DAY SUBACUTE ORAL TOXICITY STUDY

ALBINO RATS

FINAL SACRIFICE

ORGAN WEIGHT AND RATIO DATA

SUMMARY OF MEAN VALUES

ORGAN - KIDNEYS

DIETARY LEVEL (PPM)	ORGAN WEIGHT (GM)		ORGAN/BODY WEIGHT RATIO (GM/100 GM)	
	MALES	FEMALES	MALES	FEMALES
NONE	0.000	2.245	0.0000	0.7676
300	0.000	2.194	0.0000	0.7226
1000	0.000	2.170	0.0000	0.7214

NO STATISTICALLY SIGNIFICANT TREATMENT EFFECTS FOUND.

TABLE IV

TEST MATERIAL: LEVN-LITE

90-DAY SUBACUTE ORAL TOXICITY STUDY

ALBINO RATS

FINAL SACRIFICE

ORGAN WEIGHT DATA - KIDNEYS

FEMALES

GROUP	DIETARY LEVEL (PPM)	RAT NUMBER	ORGAN WEIGHT (GM)	ORGAN/BODY WEIGHT RATI (GM/100 GM)
C	NONE	1	1.780	0.71200
		2	2.430	0.69034
		3	2.280	0.76254
		4	2.150	0.82061
		5	2.540	0.73837
		7	2.050	0.68561
		8	2.340	1.01298
		9	2.140	0.67936
		10	2.340	0.87969
		11	2.380	0.76527
		12	2.140	0.73539
		13	2.420	0.74461
		14	2.310	0.72413
		15	2.140	0.79553
T-1	300	46	2.510	0.80448
		47	1.900	0.65068
		48	1.750	0.67567
		49	2.320	0.70090
		50	1.980	0.70212
		52	2.050	0.82661
		53	1.720	0.55128
		54	2.590	0.78484
		55	2.270	0.72990
		56	2.330	0.75404
		57	2.210	0.67173
		58	2.490	0.74550
		59	2.280	0.74267
		60	2.320	0.77591

TABLE IV CONTINUED

TEST MATERIAL: LEVN-LITE

90-DAY SUBACUTE ORAL TOXICITY STUDY

ALBINO RATS

FINAL SACRIFICE

ORGAN WEIGHT DATA - KIDNEYS

FEMALES

GROUP	DIETARY LEVEL (PPM)	RAT NUMBER	ORGAN WEIGHT (GM)	ORGAN/BODY WEIGHT RATI (GM/100 GM)
T-II	1000	91	2.370	0.70958
		92	1.860	0.70188
		93	2.360	0.81099
		94	1.880	0.69117
		95	2.330	0.76393
		96	2.280	0.71249
		97	2.560	0.78048
		98	1.990	0.75378
		99	2.430	0.73860
		100	2.180	0.66060
		101	2.020	0.62928
		102	2.200	0.68965
		103	1.630	0.72123
		104	2.300	0.73717

PROJECT NO. BTL-73-38
REPORT FILE

Industrial **BIO-TEST** *Laboratories, Inc.*
1810 FRONTAGE ROAD
NORTHBROOK, ILLINOIS 60062

4.

REPORT TO

MONSANTO COMPANY

EVALUATION OF KIDNEY TISSUES FROM RATS AND DOGS FED LEVN-LITE FOR 90 DAYS

BTL NO. 73-38

FEBRUARY 21, 1974

IBT NO. 661-04633

Industrial **BIO-TEST** *Laboratories, Inc.*
1810 FRONTAGE ROAD
NORTHBROOK, ILLINOIS 60062

February 21, 1974

Dr. Paul L. Wright
Monsanto Company
800 North Lindbergh Boulevard
St. Louis, Missouri 63166

Dear Dr. Wright:

Re: IBT No. 661-04633 - Evaluation of Kidney Tissues from Rats and Dogs Fed LEVN-LITE for 90 Days - BTL No. 73-38

We are submitting herewith our laboratory report dated February 21, 1974, prepared in connection with the above study.

Very truly yours,

J. C. Calandra
President

JCC:bp

REPORT TO

MONSANTO COMPANY

EVALUATION OF KIDNEY TISSUES FROM RATS AND DOGS FED LEVN-LITE FOR 90 DAYS

BTL NO. 73-38

FEBRUARY 21, 1974

IBT NO. 661-04633

I. Introduction

This report presents the results of the histopathologic examination of sections of kidneys from rats fed LEVN-LITE at dietary levels of 300, 1,000, 3,000, 10,000 or 30,000 ppm and from dogs fed 3,000, 10,000 or 30,000 ppm in their diets for 90 days (1, 2 and 3). This comprehensive histopathologic study of rat and dog kidneys was conducted to more thoroughly describe and to evaluate the toxicological significance of the renal microconcretions that were observed in the female rats (1 and 2).

Microconcretions have been observed in the kidneys of rats fed high levels of a number of inorganic phosphate salts (4, 5 and 6); similar microconcretions are also known to occur spontaneously. The highest frequency of spontaneous occurrence of this lesion is found in the female rat; incidences as high as 70% have been observed among untreated female rats in this laboratory. No deleterious effect of these microconcretions on renal function has been determined.

II. Summary

Histopathologic examinations of microconcretions present in the kidneys of female rats fed various dietary levels of LEVN-LITE demonstrate that they are identical in appearance to those arising spontaneously among untreated female rats of similar age which have been maintained under identical conditions. These microconcretions are present in the lumina of tubules located primarily at the corticomedullary junction and involve one or both kidneys. Similar microconcretions were occasionally noted in a few tubules located in the cortex and/or medulla. In the initial stage of this lesion, there is focal to diffuse degeneration and eventual necrosis of the tubular epithelium lining isolated tubules which subsequently becomes mineralized. In the latter stages of the lesion, there are small concretions within the lumina of affected tubules composed of light to dark blue concentric rings of amorphorus to granular material with a laminated appearance.

There was a greater incidence and an increased number and size of intra-tubular microconcretions in the kidneys of female rats given 10,000 or 30,000 ppm than those of spontaneous origin observed in control rats. Even though the incidences observed in other groups of treated female rats may have been slightly higher than that of their contemporary controls, they were not outside the range observed among control females. No significance is given to the microconcretions found in the female rats fed 300, 1,000 or 3,000 ppm. Renal microconcretions were not present in any of the male rats or in either male or female dogs, even at dietary levels of 3,000, 10,000 or 30,000 ppm.

It is concluded that the female rat is uniquely sensitive to the development of renal microconcretions. Furthermore, the spontaneous incidence of this lesion in the female rat can be increased by feeding high levels of inorganic phosphate. The significance of this finding is highly questionable because it was not associated with any evidence of impaired renal function or any other toxicologic manifestation. Furthermore, neither male rats nor male or female dogs exhibited any evidence of this lesion even at dietary levels as high as 30,000 ppm.

Respectfully submitted,

INDUSTRIAL BIO-TEST LABORATORIES, INC.

Report prepared by:

Donovan E. Gordon, D.V.M., Ph.D.
Diplomate, American College of
Veterinary Pathologists

Report approved by:

M. L. Keplinger, Ph.D.
Manager, Toxicology

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III. Procedure

A. Experimental Material

The kidney tissues evaluated in this study were from Charles River strain albino rats or purebred beagle dogs utilized in three previously reported studies (1, 2 and 3). The organization of the individual experiments and the dose used are shown in Table I.

TABLE I

TEST MATERIAL: LEVN-LITE

90-Day Subacute Oral Toxicity Study

Outline of Experiments

Experiment Reference	Species	Group	No. of Animals Male	Female	Dietary Level ppm
1	rat	C-I	-	15	none
2	rat	C-II	15	15	none
1	rat	T-I	-	15	300
1	rat	T-II	-	15	1,000
2	rat	T-III	15	15	3,000
2	rat	T-IV	15	15	10,000
2	rat	T-V	15	15	30,000
3	dog	C-III	4	4	none
3	dog	T-VII	4	4	10,000
3	dog	T-VIII	4	4	30,000

B. Histologic Technique

Transverse sections, approximately 4 mm in thickness, were taken from both kidneys of all experimental animals immediately after sacrifice and fixed in buffered 10% neutral buffered formalin. Paraffin-embedded sections of kidney were cut at 4-6 microns, stained with hematoxylin and eosin and examined by light microscopy.

Photomicrographs of representative renal lesions from control and test animals were prepared.

IV. Results

Table II summarizes the results of microscopic examination of the kidney tissue from the control animals and those fed LEVN-LITE.

Microconcretions were observed spontaneously only in the female rats. No renal microconcretions were observed in the kidney sections from control or treated male rats or from either male or female dogs of any treatment group. From a review of the data it can be seen that there is a definite treatment and dose related increase in the incidence and relative severity of renal microconcretions among female rats from the higher treatment levels. No treatment related effects were observed in kidney tissue from either the male rats or male or female dogs, even at the highest treatment level.

In the female rats, the microconcretions are present in the lumina of tubules located principally at the corticomedullary junction of one or both kidneys of animals from both the control and treatment groups. The localization of the lesion to tubules in this area of the kidney corresponds to that area where the terminal portion of the proximal convoluted tubules are found. Occasional microconcretions were noted in a few tubules located in the cortex and/or medulla. Various stages were noted in the development of these microconcretions. In the initial stage of their development, there is focal to diffuse degeneration and necrosis of the renal tubular epithelium lining isolated tubules. The cellular debris resulting from the degenerative process appears to serve as a nidus in the initial formation of the typical microconcretion. The cellular debris appears to undergo mineralization (dystrophic calcification) just prior to or shortly after extrusion into the

lumen of affected tubules. Several small microconcretions containing a central nucleoid of mineralization can be identified in some of the affected tubules. As these structures enlarge, the mineralization process proceeds towards the periphery of the debris. Eventually these structures coalesce and completely occlude the tubules with solid masses of calcareous material. There is a complete absence of epithelial cells lining these tubules. Many of these tubules are greatly dilated or distended with the calcereous material. In the end stage of development, the concretions are composed of light to dark blue concentric rings of amorphorus to granular material which has an "onion skin" or laminated appearance. These structures, due to their mineral content, usually shatter upon sectioning of the tissue, which produces tearing and distortion artefacts in the immediate area of the lesion. The kidneys of the test animals from the highest treatment group contain variable numbers of cortical tubules containing atypical regeneration of the tubular epithelium and in some animals, there are foci of chronic inflammation. Some of these lesions were located adjacent to tubules with microconcretions while others occurred distant to the concretions. Scattered foci of interstitial lymphoid infiltrations were also present in the kidney sections of some of the control and test animals. The microconcretions graded as +3 or greater microscopically were clearly evident upon macroscopic examination of hematoxylin and eosin stained sections and appeared as numerous dark foci or a continuous dark band located at the junction of the cortex and the medulla.

The addition of 30,000 ppm (3%) LEVN-LITE to the commercial stock added approximately 0.8% available phosphorous to the diet. This amount is equal

to slightly over 2 times the amount of available phosphorous normally present in the stock ration fed the rats. Even at this highest level of intake (over 3 times the normal phosphorous requirement) and in those animals with the greatest incidence and severity of kidney microconcretions there was no clinical pathologic evidence of impaired renal function. Kidney weight and kidney to brain weight ratios for female rats fed 30,000 ppm were nearly identical to those of the control. There was a slight increase in the kidney to body weight ratio in this group, resulting from a slightly lower body weight at autopsy (2). Absolute kidney weights and ratios did not differ between the respective control and any other treatment group (1, 2 and 3). Renal and liver functions, as measured by various blood and urine parameters (serum alkaline phosphatase, serum glutamic-pyruvic transaminase, serum glutamic oxaloacetic transaminase, blood urea nitrogen, blood glucose, glucose, urine albumin, urine pH, urine specific gravity, and urine micro-particulates) was normal for all animals examined.

In summary, the typical histologic appearance of the kidneys from the female rats consisted of a dose related increase in the incidence, number, and size of intra-tubular microconcretions in various stages of development. The histologic features of this lesion are consistent with those reported in rats following ingestion of excess inorganic phosphate incorporated in the diet (4, 5 and 6). There was no evidence of spontaneous or treatment induced microconcretions in either male rats or male or female dogs, even at dietary levels as high as 30,000 ppm. As a result of the marked sex and species differences in the development of the renal microconcretions, the relative significance of this finding in female rats is highly questionable.

TABLE II

TEST MATERIAL: LEVN-LITE

90-Day Subacute Oral Toxicity Study - Albino Rats and Beagle Dogs

Individual Histopathologic Findings in Kidneys

Species	Group	Dietary Level (ppm)	Animal Number and Sex	Microconcretions: Unilateral	Microconcretions: Bilateral	Microconcretions: Relative Number in each Kidney Section*	Microconcretions: Relative Size in affected Kidney(s)**	Focal Interstitial Lymphoid Infiltrations In Cortex or Medulla+	Focal Tubular Nephrosis associated with Regeneration of Tubular Epithelium+	Chronic Focal Nephritis+	Reference
Rat	C-I	None	1-F								1
			2-F	X		++	+				
			3-F								
			4-F								
			5-F	X		+	+				
			7-F								
			8-F		X	+++/+++++	+ to ++				
			9-F								
			10-F								
			11-F								
Rat	C-II	None	16-F					+1 (unilateral)			2
			17-F								
			18-F								
			19-F	X		+	+				
			20-F					+1 (unilateral)			
			21-F					+1 (unilateral)			
			23-F								
			24-F								
			26-F								
			27-F								
			1-M					+1			2
			2-M					+1			
			3-M								
			4-M								
			5-M								
			6-M								
			7-M								
			8-M								
			9-M					+1			
			10-M								

TABLE II continued

TEST MATERIAL: LEVN-LITE

90-Day Subacute Oral Toxicity Study - Albino Rats and Beagle Dogs

Individual Histopathologic Findings in Kidneys

Species	Group	Dietary Level (ppm)	Animal Number and Sex	Microconcretions: Unilateral	Microconcretions: Bilateral	Microconcretions: Relative Number in each Kidney Section*	Microconcretions: Relative Size in affected Kidney(s)**	Focal Interstitial Lymphoid Infiltrations In Cortex or Medulla+	Focal Tubular Nephrosis associated with Regeneration of Tubular Epithelium+	Chronic Focal Nephritis+	Reference
Rat	T-I	300	76-F								1
			77-F	X		+	+				
			78-F		X	+/+	+				
			80-F								
			81-F								
			82-F								
			84-F								
			85-F								
			86-F								
			87-F	X		+	+				
Rat	T-II	1,000	91-F		X	++/++	+ to ++				1
			92-F		X	+++/++++	+ to +++				
			93-F	X			+				
			94-F		X	+	+				
			95-F		X	+/+	+				
			96-F		X	+/+	+				
			97-F		X	++/++	+ to ++				
			98-F								
			99-F								
			103-F	X		+	+				
Rat	T-III	3,000	226-F	X							2
			227-F								
			228-F								
			229-F		X						
			230-F								
			231-F		X				+2		
			232-F								
			233-F								
			234-F								
			235-F								
			236-F								
			237-F								
			238-F		X						
			239-F					+2			
			240-F								

TABLE II continued

TEST MATERIAL: LEVN-LITE

90-Day Subacute Oral Toxicity Study - Albino Rats and Beagle Dogs

Individual Histopathologic Findings in Kidneys

Species	Group	Dietary Level (ppm)	Animal Number and Sex	Microconcretions: Unilateral	Microconcretions: Bilateral	Microconcretions: Relative Number in each Kidney Section*	Microconcretions: Relative Size in affected Kidney(s)**	Focal Interstitial Lymphoid Infiltrations In Cortex or Medulla+	Focal Tubular Nephrosis associated with Regeneration of Tubular Epithelium+	Chronic Focal Nephritis+	Reference
Rat	T-IV	10,000	256-F								2
			257-F		X	+/+	+ to ++				
			258-F			+	+				
			259-F		X	+++/++++	+ to ++				
			260-F								
			261-F		X	+/+	+ to ++				
			262-F								
			263-F		X	+++/+++	+ to +++				
			264-F								
			265-F					+1 (bilateral)			
			266-F						+1 (unilateral)		
			267-F		X	+/+	+ to ++				
			268-F		X	+++++/+++++	+ to ++++				
			269-F		X	+++++/+++++	+ to ++++				
			270-F		X	+/++	+ to +++				
			241-M								
			242-M								
			243-M								
			244-M								
			245-M								
			246-M								
			247-M								
			248-M								
			249-M								
			250-M								

TABLE II continued

TEST MATERIAL: LEVN-LITE

90-Day Subacute Oral Toxicity Study - Albino Rats and Beagle Dogs

Individual Histopathologic Findings in Kidneys

Species	Group	Dietary Level (ppm)	Animal Number and Sex	Microconcretions: Unilateral	Microconcretions: Bilateral	Microconcretions: Relative Number in each Kidney Section*	Microconcretions: Relative Size in affected Kidney(s)**	Focal Interstitial Lymphoid Infiltrations in Cortex or Medulla+	Focal Tubular Nephrosis associated with Regeneration of Tubular Epithelium+	Chronic Focal Nephritis+	Reference
Rat	T-V	30,000	286-F		X	++/++	+ to +++				2
	T-V		287-F		X	+++++/+++++	+ to +++	+1 (bilateral)	+1 (bilateral)		
			288-F		X	+++++/+++++	+ to ++++		+2 (bilateral)		
			289-F		X	+++/+++	+ to +++	+1 (bilateral)			
			290-F		X	+++++/+++++	+ to ++++	+1 (bilateral)	+1 (bilateral)		
			291-F		X	+++++/+++++	+ to ++++	+1 (bilateral)	+1 (bilateral)		
			292-F		X	+++/+++	+ to ++++	+1 to +2 (bilateral)			
			293-F								
			294-F		X	++/++	+ to +++				
			295-F		X	++++/++++	+ to +++	+2 (bilateral)			
			296-F		X	+++/+++++	+ to ++++				
			297-F		X	+++/++++	+ to +++				
			298-F		X	+++++/+++++	+ to ++++				
			299-F		X	++++/+++++	+ to +++				
			300-F		X	+/++	+ to ++				
			271-M								
			272-M								
			273-M								
			274-M								
			275-M								
			276-M								
			277-M								
			278-M								
			279-M								
			280-M								

TABLE II continued

TEST MATERIAL: LEVN-LITE

90-Day Subacute Oral Toxicity Study - Albino Rats and Beagle Dogs

Individual Histopathologic Findings in Kidneys

Species	Group	Dietary Level (ppm)	Animal Number and Sex	Microconcretions: Unilateral	Microconcretions: Bilateral	Microconcretions: Relative Number in each Kidney Section*	Microconcretions: Relative Size in affected Kidney(s)**	Focal Interstitial Lymphoid Infiltrations In Cortex or Medulla*	Focal Tubular Nephrosis associated with Regeneration of Tubular Epithelium*	Chronic Focal Nephritis*	Reference
Dog	UC	None	5-F								3
			6-F								
			7-F								
			8-F								
			1-M								
			2-M								
			3-M								
			4-M								
Dog	T-I	3,000	13-F								3
			14-F								
			15-F								
			16-F								
			9-M								
			10-M								
			11-M								
			12-M								
Dog	T-II	10,000	21-F								3
			22-F								
			23-F								
			24-F								
			17-M								
			18-M								
			19-M								
			20-M								

TABLE II continued

TEST MATERIAL: LEVN-LITE

90-Day Subacute Oral Toxicity Study - Albino Rats and Beagle Dogs

Individual Histopathologic Findings in Kidneys

Species	Group	Dietary Level (ppm)	Animal Number and Sex	Microconcretions: Unilateral	Microconcretions: Bilateral	Microconcretions: Relative Number in each Kidney Section*	Microconcretions: Relative Size in affected Kidney(s)**	Focal Interstitial Lymphoid Infiltrations In Cortex or Medulla+	Focal Tubular Nephrosis associated with Regeneration of Tubular Epithelium+	Chronic Focal Nephritis+	Reference
Dog	T-III	30,000	29-F								3
			30-F								
			31-F								
			32-F								
			25-M								
			26-M								
			27-M								
			28-M								

Symbols

*
+ = less than 10
++ = 10-30
+++ = 31-50
++++ = 51-75
+++++ > 75

**
+ = 2.5-7.5 μ
++ = 8.0-12μ
+++ = 13.0-18μ
++++ > 18 μ

\+
1 = trace or minimal in severity
2 = mild in severity
3 = moderate in severity
4 = marked in severity
5 = extreme in severity

X = Present

Legends to Figures for Study Number 661-4633

Fig. 1 Section of Kidney fron Control Rat (No. 2) with a few small mineralized microconcretions within tubules located at corticomedullary junction, X12

Fig. 2 Higher magnification of Kidney from control animal in Fig. 1, with partially mineralized microconcretions involving two adjacent tubules, X5

Fig. 3 High magnification of Kidney from animal in Fig. 1, with a large microconcretion in one tubule which is almost completely mineralized, X500

Fig. 4 Section of Kidney from Control Rat (No. 5), with a solitary intra-luminal microconcretion in an early stage of formation which contains a central nucleoid of mineralization, X500

Fig. 5 Section of Kidney from Control Rat (No. 8), showing numerous mineralize microconcretions, of various sizes, in tubules located at the corticomedullary junction. Note shattering of some of these bodies and distortion of adjacent tubules, X125

Fig. 6 Higher magnification of a microconcretion from animal in Fig. 5, X500

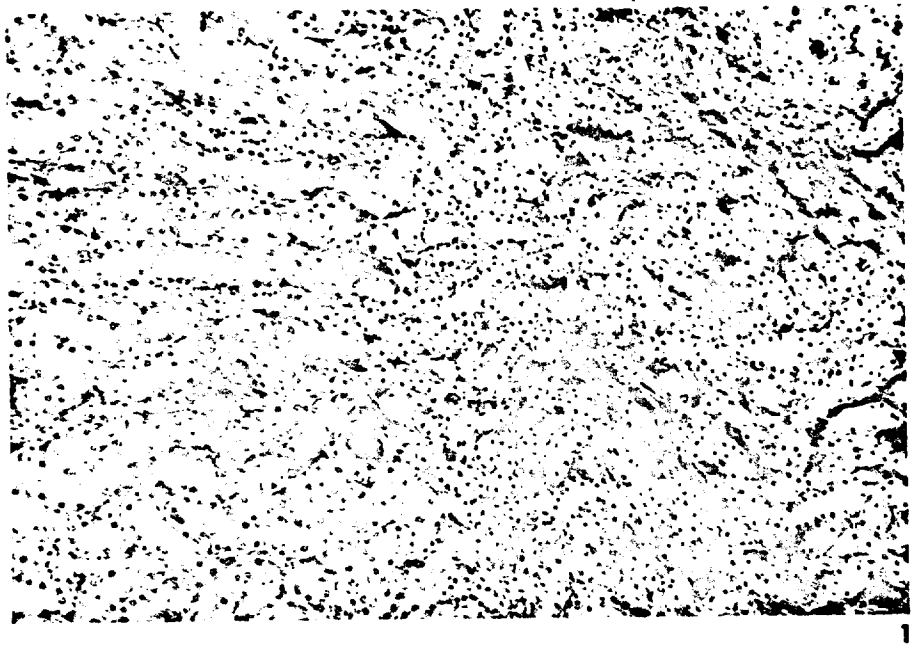
Fig. 7 Section of Kidney from Test Rat (No. 259, Group IV), with several mineralized microconcretions within lumina of tubules located at corticomedullary junction. Portions of some of these structures shattered during sectioning and empty spaces remain where they were present, X125

Fig. 8 Section of Kidney from Test Rat (No. 269, Group IV), with multiple mineralized microconcretions within lumina of tubules, X125

Fig. 9 Higher magnification of a portion of one microconcretion shown in Fig. 8. Note laminated appearance of these structures and normal appearing tubules adjacent to lesions, X500

Fig. 10 Section of Kidney from Test Rat (No. 92, Group IV), note various stages o development and mineralization of microconcretions. There are several individual microconcretions within the large tubule in the center of the field which will eventually coalesce and completely occlude the lumen. Only a few viable epithelial cells can be seen along the basement membrane of this tubule, X500

Fig. 11 Section of Kidney from Test Rat (No. 288, Group V), with a small focus of chronic interstitial inflammation (arrow) adjacent to tubules with mineralized microconcretions, X125



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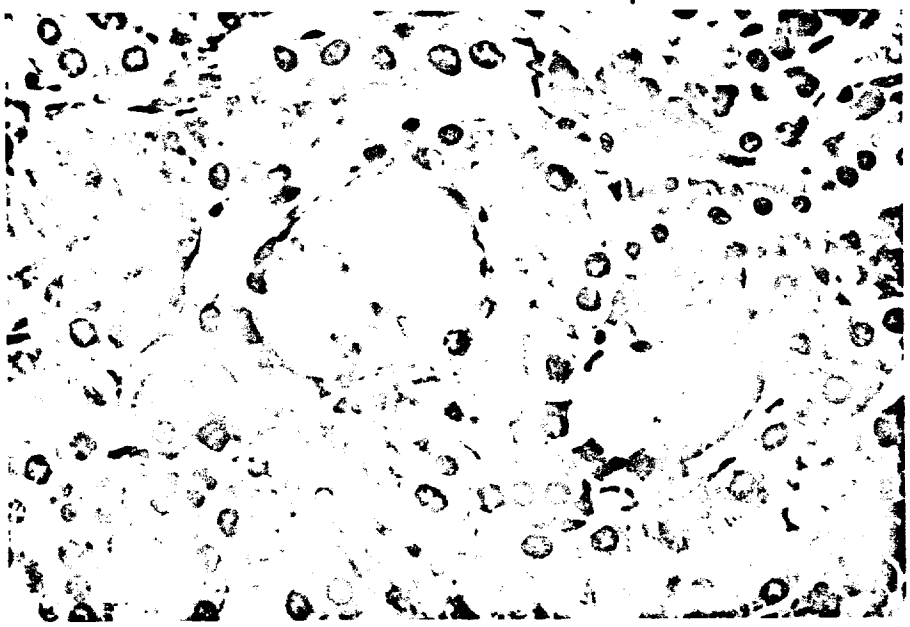

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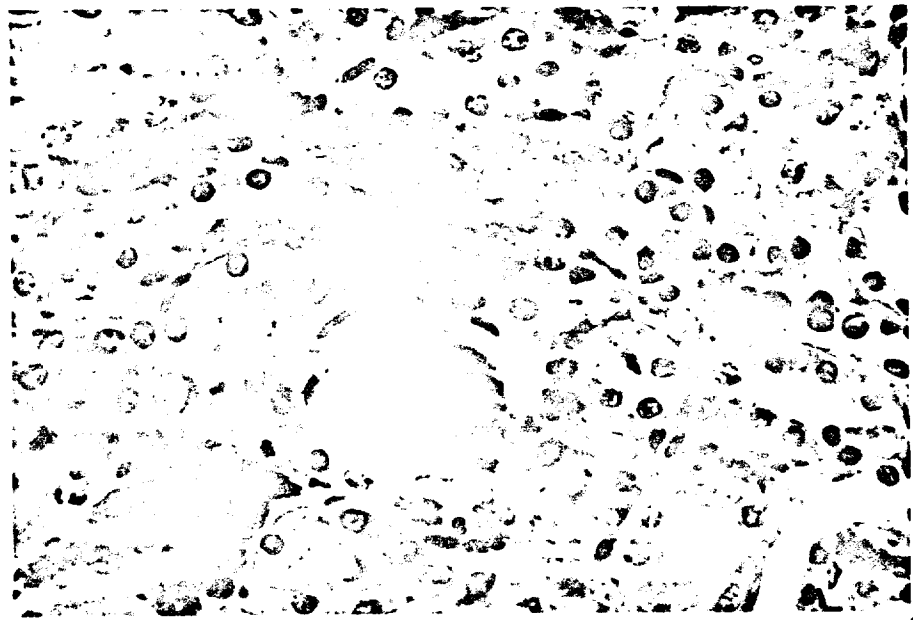

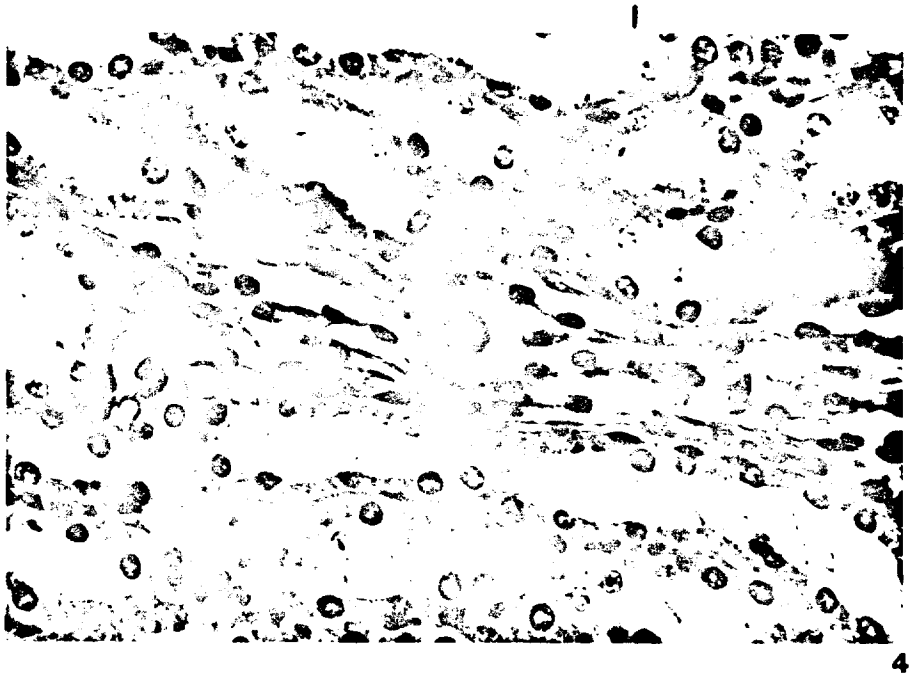

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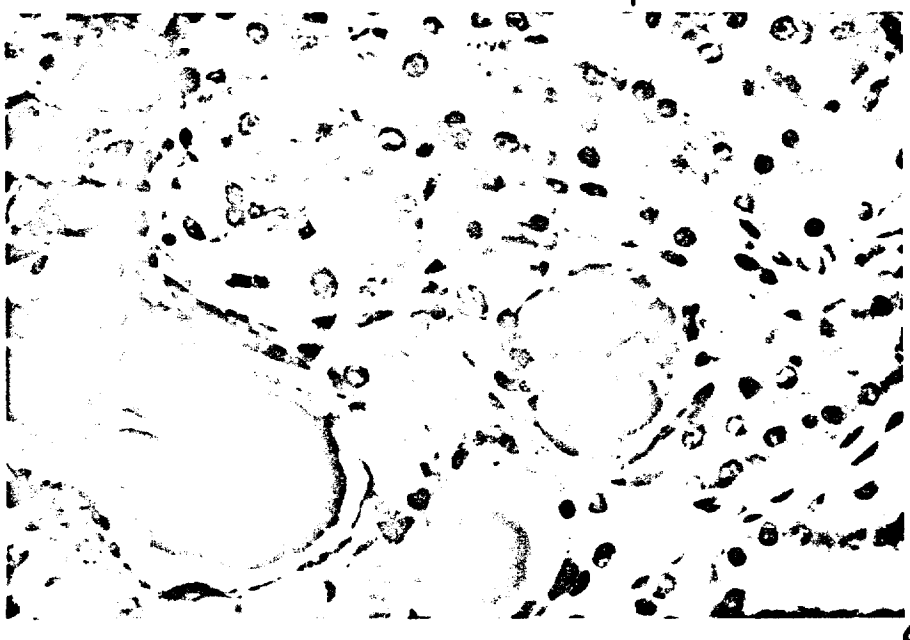

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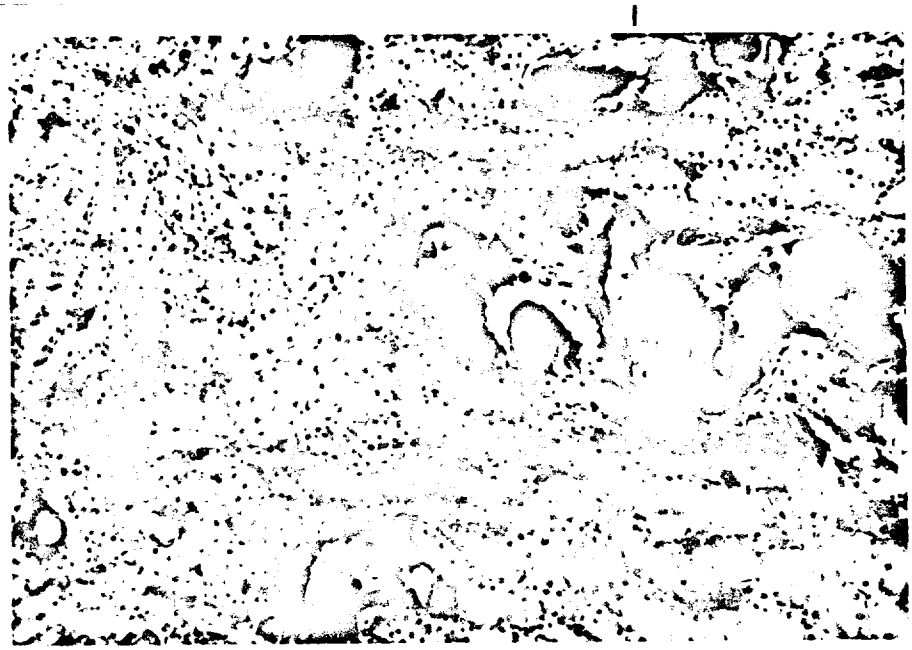

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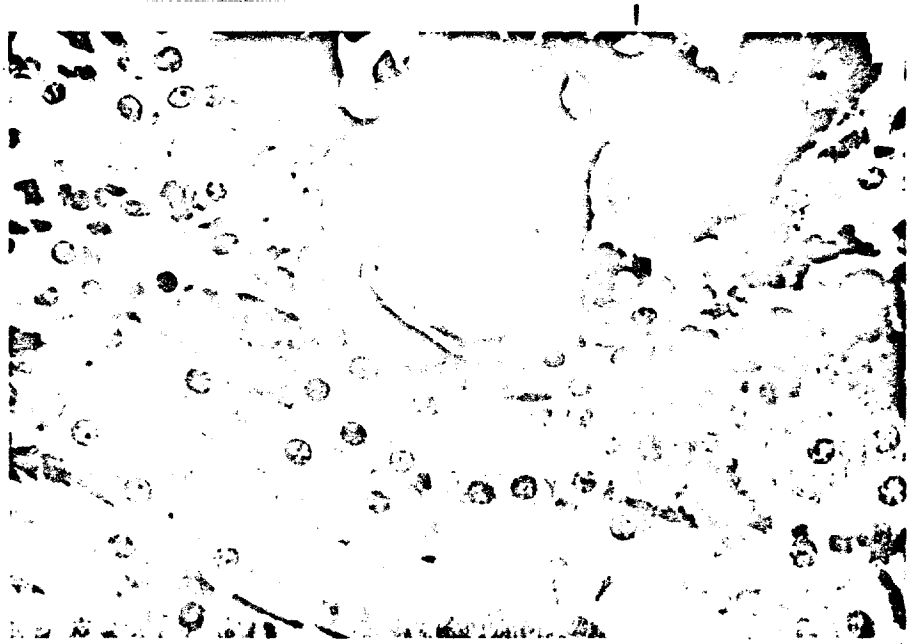

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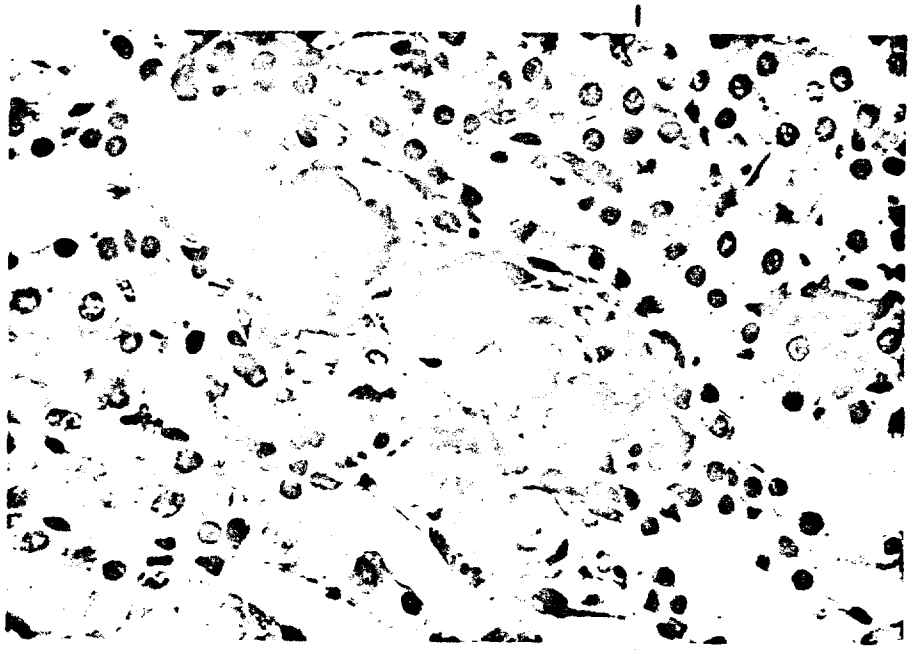

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2. Ninety-day Subacute Oral Toxicity Study with LEVN-LITE in Albino Rats. Industrial BIO-TEST Laboratories, Inc. Report No. B747. June 28, 1972.

3. Ninety-day Subacute Oral Toxicity Study with LEVN-LITE in Beagle Dogs. Industrial BIO-TEST Laboratories, Inc. Report No. J749, June 21, 1972.

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6. Toxicity Studies on Phosphates:

   I. Short-term oral toxicity of condensed phosphates in rats and dogs.

   II. Chronic oral toxicity studies of sodium tripolyphosphate in rats.

   III. Chronic oral toxicity of sodium trimetaphosphate in rats.

   IV. Chronic oral toxicity of sodium hexametaphosphate in rats.

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